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TABLE OF CONTENTS, VOLUME 20, 1933

INTUMESCENCES ON POPLAR LEAVES. I. STRUCTURE AND DEVELOPMENT [1]

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INTRODUCTION

The peculiar wart-like outgrowths of cells on leaves and stems, which were named " intumescences " by Sorauer (12), have been observed on a number of plants. Sorauer himself found them on more than a dozen plants, and other workers have recorded their occurrence on plants of various families. They have been found on both monocotyledonous and dicotyledonous angiosperms, on gymnosperms, and even on ferns. Viala and Pacottet (14) found them on the cultivated grape; Dale (2) on *Hibiscus vitifolius;* Steiner (13) on *Ruellia formosa,* and *Aphelandra porteana;* Atkinson (1) on the apple, and the tomato; Douglas (3) on the potato; and Von Schrenk (15) on the cauliflower. Cabbage plants have been observed to develop intumescences under differing conditions by Harvey (5), and Wolf (17). Schilling (11) coated twigs of a number of plants with paraffin, or vaseline, with the result that intumescences were formed. Hahn, Hartley, and Rhoades (4) observed such growths on roots of several species of conifers. Küster (6, 7) studied the intumescences on leaves of *Populus tremula,* and on the pods of *Pisum sativum, Vicia faba,* and other leguminous plants; and Wallace (16) investigated the intumescences produced by the action of ethylene gas on apple twigs. Numerous other examples of these abnormalities are cited in the literature, which is reviewed by Küster (8).

In connection with a study of regeneration the author (9) discovered intumescences on leaves of *Populus tremuloides,* and *P. grandidentata,* in which he was trying to induce renewed growth. He has found these growths also on leaves of *Eucalyptus cornuta,* of *E. coccifera,* and of *Thurberia* sp. grown in the greenhouse, and on injured leaves of *Mitchella repens* (10) and of *Hieracium venosum.*

In connection with the work on regeneration mentioned above, leaves of a number of species of plants were cut into pieces and placed on plates of nutrient agar, or floated on nutrient solutions, to test their capacities for regeneration. Most of these trials were without result so far as any outgrowth was concerned, but it was noticed that the leaves of *Populus grandidentata* soon

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[1] Paper No. 373 from the Dept. of Botany, University of Michigan. A part of this work was done at the University of Michigan Biological Station.

produced growths on the surfaces next to the agar, or the nutrient solution. These were thought, at first, to be evidences of regeneration, but it soon became apparent that they were incapable of continued growth, and were intumescences.   Shortly after these growths were found on leaves of *P. grandidentata*, similar ones were discovered on leaves of *P. tremuloides*.   Repeated trials showed that the growths could be developed at will, not only on agar plates and on nutrient solutions, but also when the leaf pieces were floated on water in a closed chamber.   Next, it was found that whole leaves of these two species of poplar would throw out intumescences without being in contact with an agar surface or a water surface, if they were only enclosed in an air-tight chamber.   It did not seem to make any difference whether the leaves were removed from the twigs, were left on the twigs, or were left attached to the trees.   However, some differences in the rate of development were found under differing conditions, and the causes of these outgrowths were investigated in some detail.   The details of these studies and their results will be presented in a later paper.

INTUMESCENCES ON POPLAR LEAVES IN NATURE

After the discovery of the artificially induced intumescences on poplar leaves a search was made for such abnormalities in nature.   They were never found on leaves fully exposed to the air, a result that might have been predicted from other studies on such growths on other species.   An abundance of moisture has been found necessary to the formation of such abnormalities in most species, and in the open air such a supply of moisture is rarely encountered.   However, various species of insects roll the leaves of plants to form chambers for the development of their larvae, and such chambers were found on both of the poplar species concerned.   Wherever the leaves were rolled tightly narrow chambers were formed to which the access of the dry outer air was somewhat restricted, and in these chambers intumescences were found.   Sometimes only a very small number of intumescences would be found on the leaf, and considerable areas of leaf would occur from which the mesophyll had been eaten by the insect larvae.   It is obvious that the breaking of the epidermis by the long, abnormal cells of the outgrowth gives an easy access to the soft mesophyll cells, which the larvae can attack at once without the necessity of gnawing through the relatively tougher epidermis. It is not too much to suppose that these insects have come to depend, to a limited degree, on this reaction of the leaf to the unusual condition presented by the rolling of the lamina; although, since the larvae can and do eat the epidermis, it is possible that the advantages offered in the form of intumescences are of no consequence to them.

Certain insects make webs by which they fasten leaves of *Populus grandidentata* together in pairs.   Wherever these leaves were pressed close together in forming a larval chamber conditions favorable to intumescences

were supplied, and usually intumescences were found in such chambers. Here, too, the larvae consumed the cells of the intumescences, and if the larvae were near maturity when found, only a few small outgrowths around the outside of the areas attacked by the larvae would be seen.

Leaves of *P. tremuloides* were fastened together in the same way as those of *P. grandidentata*, but whether by the same species of insect or not, has not been determined. So far as the outgrowths were concerned the conditions in the two species of poplar were identical.

Aside from the examples of insect attack, no intumescences have ever been found on normal poplar leaves. It is unusual for the conditions which are needed for the inception of such outgrowths to be supplied by the normal environment of the plant, which may be considered fortunate for the health of the species concerned; otherwise the abnormal lesions of the epidermis would give entry to destructive organisms with great damage to the leaves as a possible result.

SPECIES STUDIED

No species of *Populus* other than the two already mentioned has been found on which intumescences will develop, under any of the conditions which have been tried. Repeated attempts to induce them on *P. deltoides, P. balsamifera, P. alba, P. simonii,* and *P. nigra* var. *italica* were without result.

It seems curious that two species of *Populus* should behave so differently from the others, but the conditions which give rise to intumescences in these species do not have the same effect on most other kinds of plants. In the attempt to see whether plants of other families and genera would react in like manner the following species were enclosed in damp chambers; in all cases except one, quite without result:

Polypodiacae
    Pteris aquilina
    Polystichum lonchitis
    Aspidium thelypteris
    Cystopteris bulbifera
Osmundaceae
    Osmunda regalis
Ophioglossaceae
    Botrychium simplex
    Botrychium virginianum
Equisetaceae
    Equisetum arvense
    Equisetum sylvaticum
    Equisetum laevigatum
    Equisetum hyemale
    Equisetum scirpoides
Lycopodiacceae
    Lycopodium lucidulum
    Lycopodium clavatum

    Lycopodium obscurum var. *dendroideum*
Isoetaceae
    Isoetes sp.
Taxaceae
    Taxus canadensis
Pinaceae
    Sequoia sempervirens
    Pinus resinosa
    Larix laricina
    Picea mariana
    Abies balsamea
    Tsuga canadensis
    Thuya occidentalis
    Juniperus communis
    Juniperus horizontalis
    Juniperus virginiana
    Cupressus lawsoniana
Typhaceae
    Typha latifolia

Gramineae
Phleum pratense
Agropyron repens
Cyperaceae
Eleocharis rostellata
Araceae
Symplocarpus foetidus
Acorus calamus
Commelinaceae
Tradescantia virginiana
Commelina bangalensis
Liliaceae
Aloe ciliaris
Erythronium americanum
Clintonia borealis
Smilacina racemosa
Maianthemum canadense
Polygonatum biflorum
Smilax herbacea
Iridaceae
Iris versicolor
Iris lacustris
Orchidaceae
Epipactis repens var. *ophioides*
Salicaceae
Salix lucida
Salix longifolia
Myricaceae
Myrica gale
Myrica asplenifolia
Juglandaceae
Juglans nigra
Betulaceae
Corylus americana
Corylus rostrata
Betula alba var. *papyrifera*
Alnus incana
Fagaceae
Fagus grandifolia
Quercus borealis
Quercus velutina
Urticaceae
Ficus rubescens
Ulmus americana
Humulus lupulus
Morus alba
Santalaceae
Comandra umbellata
Polygonaceae
Rumex acetosella
Polygonum amphibium
Fagopyrum esculentum
Chenopodiaceae
Chenopodium album

Caryophyllaceae
Lychnis alba
Saponaria officinalis
Nymphaeaceae
Nelumbo lutea
Magnoliaceae
Magnolia sp.
Cruciferae
Matthiola incana
Lepidium virginicum
Brassica nigra
Radicula aquatica
Sarraceniaceae
Sarracenia purpurea
Droseraceae
Drosera rotundifolia
Drosera longifolia
Crassulaceae
Sedum acre
Sedum spurium
Sedum allantoides
Sedum stahlii
Crassula arborescens
Cotyledon fulgens
Echeveria pulvinata
Echeveria clavaria
Bryophyllum calycinum
Bryophyllum crenatum
Saxifragaceae
Saxifraga sarmentosa
Ribes floridum
Hamamelidaceae
Hamamelis virginiana
Rosaceae
Pyrus malus
Amelanchier spicata
Crataegus sp.
Fragaria virginiana
Geum rivale
Rubus alleghiensis
Rosa sp.
Prunus serotina
Prunus virginiana
Prunus pennsylvanica
Prunus pumila
Leguminosae
Acacia riceana
Gleditsia triacanthos
Robinia pseudo-acacia
Linaceae
Reinwardtia indica
Simarubaceae
Ailanthus glandulosa

Euphorbiaceae
  Ricinus communis
Anacardiaceae
  Rhus typhina
Celastraceae
  Evonymus variegatus
  Evonymus aureus
Aceraceae
  Acer saccharum
  Acer rubrum
Melianthaceae
  Melianthus major
Balsaminaceae
  Impatiens biflora
  Impatiens sultani
Tiliaceae
  Tilia americana
Malvaceae
  Althea rosea
  Malva rotundifolia
  Hibiscus tiliaceus
  Hibiscus rosa-sinensis
Begoniaceae
  Begonia sharffiana
  Begonia peltata
  Begonia phyllomaniaca
Eleagnaceae
  Shepherdia canadensis
Lythraceae
  Decodon verticillatus
Myrtaceae
  Myrtus communis
Onagraceae
  Oenothera biennis
  Fuchsia hybrida
Araliaceae
  Hedera helix
Cornaceae
  Cornus canadensis
  Cornus circinata
  Cornus florida
  Aucuba japonica
Ericaceae
  Pyrola elliptica
  Ledum groenlandicum
  Kalmia polifolia
  Andromeda glaucophylla
  Chamaedaphne calyculata
  Gaultheria procumbens
  Arctostaphylos uva-ursi
  Gaylussacia baccata
  Vaccinium pennsylvanicum
  Vaccinium macrocarpon

Oleaceae
  Fraxinus americana
  Ligustrum japonicum
  Ligustrum vulgare
Gentianaceae
  Menyanthes trifoliata
Apocynaceae
  Vinca major
  Nerium oleander
  Apocynum cannabinum
Asclepiadaceae
  Asclepias incarnata
  Asclepias syriaca
  Hoya carnosa
Boraginaceae
  Cynoglossum officinale
Labiateae
  Coleus blumei
  Nepeta hederacea
  Stachys lanata
Solanaceae
  Solanum lycopersicum
  Solanum tuberosum
  Solanum dulcamara
  Solanum miniatum
  Nicotiana glauca
  Lycium halimifolium
Scrophulariaceae
  Antirrhinum majus
  Veronica americana
  Veronica speciosa
  Verbascum thapsus
Lentibulariaceae
  Utricularia vulgaris var. *americana*
Plantaginaceae
  Plantago major
Rubiaceae
  Mitchella repens
Caprifoliaceae
  Diervilla lonicera
  Viburnum acerifolium
  Viburnum cassinoides
  Sambucus canadensis
  Sambucus racemosa
Compositae
  Solidago sp.
  Aster laevis
  Helichrysum petiolatum
  Antennaria sp.
  Erigeron canadensis
  Lactuca canadensis
  Taraxacum officinale
  Hieracium venosum
  Kleinia articulata

The one exception mentioned was *Thuya occidentalis,* which in one case developed a single intumescence, but could not be induced to produce any more. *Mitchella repens,* with which the writer has carried on numerous experiments (10), often develops intumescences of the mesophyll when the epidermis has been removed, but unless this is done none ever appear. Many of the plants listed have been tried a number of times and it appears that the formation of intumescences on leaves enclosed in damp chambers is decidedly unusual. It is strange that tomato plants, which have been reported by Atkinson (1) to produce intumescences in atmospheres of excessive humidity, and potatoes, which according to Douglas (3) develop these growths under similar conditions, have not as yet given such results in this study. It is possible that the variety used is of importance, but neither of these writers mentioned the varieties which they studied, so one is left in doubt concerning these species.

The behavior of *Populus tremuloides* is so nearly like that of *P. grandidentata* that it is unnecessary to give a detailed account of each species. In instances where *P. tremuloides* differs notably from the more carefully studied *P. grandidentata,* the fact will be noted; otherwise agreement between the two species may be assumed.

AGE OF LEAVES

The age of the leaves which will produce intumescences seems not to be a matter of much importance. It has been found that very young leaves, just emerging from the bud, will not react in this way; but as soon as they reach about half their full size they become susceptible. At first it was thought that the heavy coating of hairs which cover the young leaves might have an effect in the process. However, these hairs remain on the leaves until they are nearly or quite mature, though of course they are more widely spaced as the leaves enlarge. Removal of the hairs from very young leaves does not bring about any change in their reaction, and their removal from half-grown leaves does not increase the number or the size of the outgrowths. It is most likely that these young leaves have been subjected in the bud to just those conditions which induce intumescences on the older leaves, and they are conditioned to such circumstances to such an extent that they do not suffer from a return to such an environment. The older leaves may have been removed from such surroundings long enough to have lost their tolerance of them, so that they react when returned to them. So little is known about such matters that anything one may say about them is mere conjecture.

Very old leaves at the end of the summer have failed to react in the trials which have been made in three successive seasons, although in several tests leaves which had developed some autumn coloring still were capable of developing intumescences of the usual type. Leaves which appear very dry, even in mid-summer, will not react in the typical way, perhaps because they are sub-normal in their physiological activities. It is possible that they are

low in carbohydrate content, though this has not been proved. Dusty leaves, found where dust from roads has settled over them, usually fail to give rise to any outgrowths.

In general leaves from all parts of the tree react in the same way, and give well-developed intumescences without regard to their position on the tree. However, the leaves produced by young root-sprouts rarely develop many intumescences, though those from young trees of a somewhat larger size give normal results.

LOCATION OF THE ABNORMAL OUTGROWTHS

The outgrowths may be found on all parts of the leaf. They are usually abundant and well-developed on the petioles whether these have laminae attached to them or not. On the laminae the growths are scattered in what appears at first to be a random fashion, but more careful observation reveals the fact that they are always in close connection with the veins or veinlets of the leaf; in fact it seems that it is very unusual for them to originate in a vein islet. The most abundant outgrowths as well as the largest ones are found alongside, or directly on the midrib and the larger veins. This is quite different from the findings of Viala and Pacottet (14), who report that they never found any intumescences on the veins of leaves of the grape.

The lower epidermis of the leaf blade usually shows the greatest number of the abnormalities, although some leaves have more on the upper epidermis than on the lower. Leaves may be seen in which there seems to be about an equal number on both surfaces, and on such leaves the number is commonly very great. It is much more usual to find a considerable number of outgrowths on the lower surface, and none on the upper, than to encounter the reverse condition.

PARTS OF LEAVES INVOLVED

Intumescences on the upper surface may involve only the upper layer of the palisade or both layers of palisade. In normal leaves of both *Populus grandidentata* and *Populus tremuloides* only two layers of palisade are present, as is shown in table 2. In no case was a larger number of layers found in these species, either in the normal leaves, or in those bearing intumescences. Neither has any case been found where an outgrowth on the upper surface involved the spongy parenchyma.

Normal leaves of *Populus grandidentata* show three, four, or rarely five layers of spongy parenchyma (table 1). The number of layers of spongy parenchyma in *Populus tremuloides* is slightly larger. Five cell layers is the most common number, but four, six, and seven layers are found. Intumescences on the lower surface may involve only the layer of cells next to the lower epidermis; they may extend to the first two layers; or they may influence all the cells of the sponge which are included in the area affected by the disturbance without affecting at all the layers of palisade. In all cases, how-

ever, the cells nearest the epidermis show the greatest enlargement. This is equally true of the cells included in the swellings on the upper surfaces of leaves.

TABLE I. *Numbers of Layers of Cells in Normal Poplar Leaves and in Leaves with Intumescences*

Species	Condition of Leaf	Part of Leaf	Number of Counts	Number of Layers of Cells						
				2	3	4	5	6	7	8
Populus grandi-dentata	Normal	Palisade	50	50	—	—	—	—	—	—
Populus grandi-dentata	Normal	Spongy parenchyma	50	—	3	40	7	—	—	—
Populus grandi-dentata	Intumescence on upper surface	Palisade	50	50	—	—	—	—	—	—
Populus grandi-dentata	Intumescence on lower surface	Spongy parenchyma	50	—	1	36	14	—	—	—
Populus tremuloides	Normal	Palisade	50	50	—	—	—	—	—	—
Populus tremuloides	Normal	Spongy parenchyma	50	—	—	9	33	6	2	—
Populus tremuloides	Intumescence on upper surface	Palisade	50	50	—	—	—	—	—	—
Populus tremuloides	Intumescence on lower surface	Spongy parenchyma	50	—	—	6	34	7	2	1

In intumescences on veins, which are usually very well developed, the outermost layer of cells is most affected, but changes may be seen in all cells outside the bundle sheaths.

The cells of the epidermis do not take part in the formation of these outgrowths. If an intumescence is small, the epidermis may be stretched by the extension of the cells inside it, but not ruptured. In the growth of large intumescences the epidermis can not be stretched enough to allow for the great increase in cell size, and it is finally torn and turned back by the protruding cells. In no case was it found that the cells of the epidermis were enlarged in the process, so the intumescences prove to be growths of the mesophyll alone.

Outgrowths on the petioles usually involve all the cells in the affected area between the epidermis and the sheaths of the vascular bundles. As is the case with the laminae of the leaves, some intumescences may appear which affect only the outermost layer of the tissue inside the epidermis, while larger ones may bring about the swelling of all the cells outside the vascular bundles. The outermost layers are always more noticeably changed than those which

lie deeper in the petiole. The epidermis is ruptured by the large intumescences, but its cells are not enlarged.

THE INTUMESCENCES AS HYPERTROPHIES

Intumescences may be *hypertrophies,* in which the original cells of the tissue are enlarged, but not increased in number as a result of the disturbance; or they may be *hyperplasias,* in which the number of cells is increased while the size of the individual cells remains the same as those of the normal tissue. Some examples appear to be mixtures of the two types of growth. The growths which were observed in this study are hypertrophies. This is proved by the failure to find any division figures, or any other evidence of renewed cell division in the leaf. Table 1 shows that the number of cell layers in the leaves is not changed by the development of the intumescences. That the size of the abnormal cells is much greater than that of normal cells is shown very clearly by table 2. The greatest cell length from an intumescence on

TABLE 2. *Lengths of Normal Cells and of Cells of Intumescences in Populus grandidentata. Measurements are Given in µ*

Type of Cell	Number of Variates	Range of Measurements	Average
Normal cells, lowest layer of spongy mesophyll .	150	6.9–17.2	10.7
Normal cells, second lowest layer of spongy mesophyll	181	6.9–24.1	14.5
Normal cells, upper layer of palisade	158	24.1–51.6	40.1
Cells of intumescences on lower surface of leaf ..	101	37.8–99.8	62.8
Cells of intumescences on upper surface of leaf...	120	41.3–68.8	57.2

the lower surface of a leaf is more than fourteen times that of the least length of normal cells of the spongy parenchyma. The longest hypertrophied cell of the upper layer of the palisade is more than seven times as long as the greatest measurement recorded in the table for normal cells from the same region. Since no attempt was made to choose the very largest intumescences for measurement of the cells, it is likely that even larger cells may be encountered in such growths.

The cells of the intumescences are not often much elongated in the plane parallel to the surface of the leaf. It is not likely that there is any polarity about the process; but probable that the cells expand most where there is most space available. In the leaves of *Populus grandidentata,* and *P. tremuloides* the spongy mesophyll is rather close in texture and does not allow much room for the expansion of any one cell. If each of the cells of the intumescence swells a little, all the space is packed tight, and further expansion must be outward. In the palisade layers, especially the upper layer, where the cells normally stand very close together, there is even less room for lateral expansion, and the cells of intumescences elongate and push out, with little or no lateral expansion.

CHANGES IN THE HYPERTROPHIED CELLS

Aside from the fact that the cells increase in size to an amazing degree, they do not undergo any striking change. They do not become separated from one another except at the outer ends where they grow out to a different extent. Here it appears that the separation is due to differential growth of
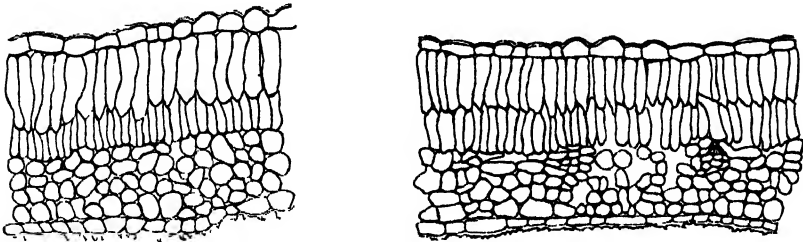
TEXT FIGS. 1 and 2. FIG. 1 (left). Section of a normal leaf of *Populus grandidentata*. FIG. 2 (right). Section of a normal leaf of *Populus tremuloides*.

the outer ends of the cells, and not to any splitting apart of the walls. In the other parts of the intumescences the cells seem to elongate simultaneously, so that there is a general growth which prevents the cells from being torn apart. Even the outer cells remain firmly attached to the others, in direct contrast to the cells in many similar types of abnormal growth.
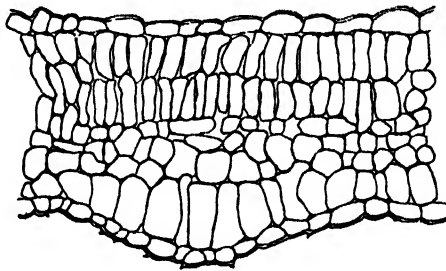
TEXT FIG. 3. Intumescence developing on lower surface of a leaf of *Populus grandidentata*, which has been kept 72 hours in a damp chamber.

The increase in cell size takes place by the growth of the walls, and there is no evidence that the walls are thinned by stretching. Neither does any sign of the corrosion or digestion of any of the layers of the cell walls appear, although detailed measurements of the thickness of walls of normal and hypertrophied cells have not been made. Careful study of the walls has failed to reveal any thinned areas, such as have been reported for intumescences in some other plants.

The enlarged cells have one or two very large vacuoles, and often the protoplasm between the two vacuoles of a cell gives an appearance of cell division, the true nature of which is discovered by more careful examination.

The layers of protoplasm along the cell walls appear to be thinned out some-
what by the great increase in cell surface, but do not appear abnormal in any
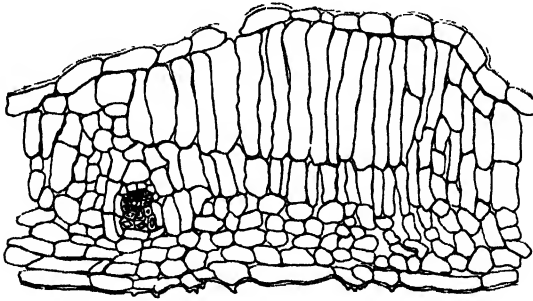
TEXT FIG. 4.   Intumescence developing on the upper surface of a leaf of *Populus
grandidentata*, which has been kept 96 hours in a damp chamber.

other respect.   The chloroplasts do not increase in number in hypertrophied
cells, and accordingly they are separated more widely than in normal cells.
Examination of the living cells shows them to be still green, although their
wide separation makes the tissue appear nearly colorless to the naked eye.
Neither in living material nor in stained sections do they appear distorted or
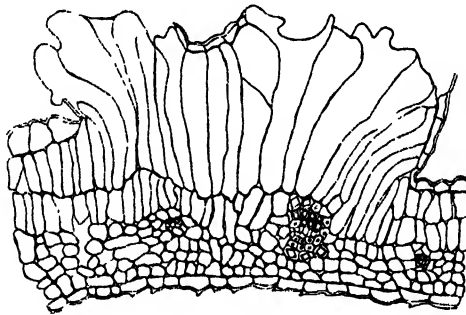
TEXT FIG. 5.   Intumescence on upper surface of a leaf of *Populus grandidentata*
after 6 days in a damp chamber.

abnormal in any way.   No crystals have been observed in these cells, though
they are common along the vascular bundles in the cells of the bundle sheaths,
in both normal leaves and those which have been enclosed in damp chambers.
It does not appear that these crystals are more numerous in the experimental
than in the normal leaves.

RATE OF DEVELOPMENT

Sections were made of leaves taken from the experimental chambers at
twenty-four-hour intervals to determine the rate of change in the cells af-
fected by the abnormal environment.   At the end of forty-eight hours no
changes of any kind could be detected (text figs. 1, 2), but after seventy-two

2

hours some cells showed considerable swelling (text fig. 3), which sometimes was more pronounced in a transverse direction than in the longitudinal one. This swelling fills up a considerable part of the air space in the spongy paren-
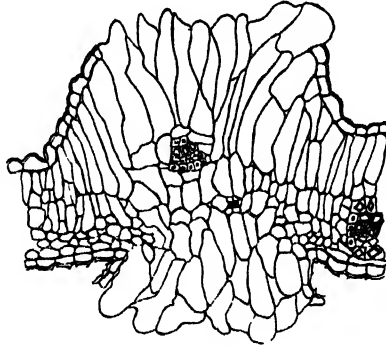
TEXT FIG. 6. Intumescences on both surfaces of a leaf of *Populus grandidentata*, which has been in a damp chamber for 6 days.

chyma, and may not cause any distension of the epidermis although in many cases the cells are more than doubled in size, and the epidermis is forced outward for a considerable distance.

After 96 hours the cells have taken up all the free space within the leaf, and have begun to thrust the epidermis outward, but the tension is usually not yet great enough to rupture it (text fig. 4).   At the end of 120 hours the
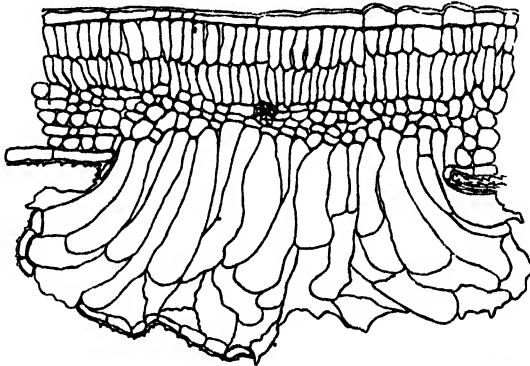
TEXT FIG. 7. Intumescence on lower surface of leaf of *Populus grandidentata,* which has been kept 9 days in a damp chamber.

epidermis shows numerous breaks through which the cells are beginning to protrude, and at this time the intumescences can be detected easily by the naked eye.

When the leaves have been in the damp chamber for 144 hours the intumescences are for the most part fully developed, although some may have been delayed in beginning their growth and are still immature (text figs. 5, 6). The epidermis has now been turned back by the swelling of the tissue inside,

and may show collapse of some of its cells.   From this time on little change takes place in the intumescence (text figs. 7, 8, 9), although there are always some which grow to an unusual size, and these will not have reached their
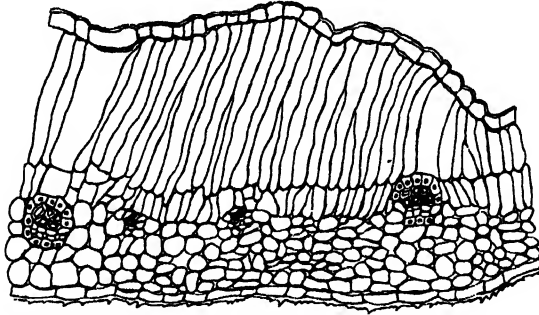
TEXT FIG. 8.   Intumescence on upper surface of a leaf of *Populus tremuloides*, which has been kept 9 days in a damp chamber.

full extension as yet.   Most of the cells appear to remain unchanged for several days after this, but by that time the leaves begin to show a general decline, or they are cut off from the twig by abscission.   In parts of leaves which have been sterilized and placed on nutrient agar, the life of the cells is considerably prolonged, and the greatest extension of intumescences has been observed on such specimens.   But under the ordinary conditions in the damp chambers, the outgrowths begin to collapse and decay as soon as the leaves begin to look unhealthy, a state which they usually reach after eight or ten days.
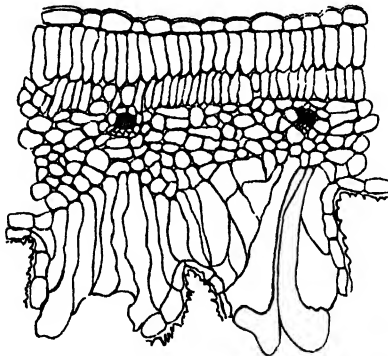
TEXT FIG. 9.   Intumescence on lower surface of a leaf of *Populus tremuloides*, which has been kept 12 days in a damp chamber.

FATE OF THE INTUMESCENCES

The outgrowths are never cut off by a periderm layer in poplar leaves. The conditions under which they develop are not conducive to the development of periderm; although Dale (2) found that the intumescences on *Hibiscus vitifolius* were sometimes cut off by a layer of periderm, presumably while subjected to a similar environment.   Whether such a result would be reached

in the open air can not be ascertained, because after the leaves have been kept in a damp chamber for a time long enough to allow intumescences to develop they are no longer able to endure the dry open air, and they speedily wilt and die.   For the same reason one can not tell whether the intumescences would dry down in the open air, although that would almost certainly occur if the leaves could be kept alive long enough.   Why the leaves should lose their ability to endure the drought of the open air, after a few days in a damp chamber, is not yet known, but the experience of the writer suggests that such a change takes place in leaves of most specimens of plants which have been subjected to such conditions.

DISCUSSION

There seems to be no law as to the development of intumescences within groups of plants.   Certain species in a genus may react in this way while related species seem immune.   Thus Küster (6) could produce outgrowths on *Populus tremula* at will, but was unable to secure them on *P. pyramidalis* and *P. candicans;* and the writer was able to cause their production on *P. grandidentata* and *P. tremuloides* and not at all on *P. deltoides, P. balsamifera, P. alba, P. simonii,* or *P. nigra* var. *italica.*   Furthermore these growths have been found in widely separated families, while intermediate families have not been shown to produce them.   Naturally, it is not likely that all possible cases have been noted, but so far as one set of environmental conditions is concerned in their formation, they have been sought in a large number of plants.   It is not always possible to secure the same results as other investigators have recorded, as was seen by Küster when he failed to cause the growth of intumescences on *Vitis* and *Epilobium,* which had been reported previously.   The writer failed to secure the results with tomato and potato, reported by Atkinson (1) and by Douglas (3).

The leaf is most often the organ on which intumescences arise, but Sorauer (12), Schilling (11), and Wallace (16), have found them on twigs; Dale (2) reported them on flowers and fruits; and Hahn, Hartley, and Rhoades (4) observed them on roots.

The part of an organ on which they develop shows a certain amount of variation in different plants.   On leaves the lamina is most often involved, but Küster (6), as well as the writer, found them on petioles.   Where they appear on the leaf lamina, they are usually seen on both upper and lower surfaces, as has been observed by Sorauer, Küster, and Wolf (17).   In this investigation they have been found on both surfaces of leaves of both species concerned (text fig. 6).   Viala and Pacottet (14), however, say that they were able to find such abnormalities on the lower surface only.   They report that no intumescences were found on the veins of the grape leaf, which is quite contrary to the findings of Sorauer, Küster, and the writer, who found them most abundant and best developed along the midribs and the large veins.

Intumescences are not always alike in structure, for they may consist en-

tirely of swollen cells, or they may be made up of cells not noticeably increased in size but greatly augmented in number. Again, they may represent a mixture of the two preceding types of tissue modification. To the first group, or hypertrophies, the intumescences on cabbage reported by Wolf belong, as well as those studied by Küster on *Populus tremula,* and by Viala and Pacottet on the grape. Those concerned in the present investigation on *Populus grandidentata* and *P. tremuloides* are certainly hypertrophies, and no increase in the number of cells has ever been found in them. Sorauer has figured hypertrophies on *Cassia tomentosa* and *Ficus elastica.*

Hyperplasias, which consist of masses of cells greatly increased in number, are common in intumescences. Steiner (13) published figures of such growths on *Ruellia formosa* and *Aphelandra porteana;* and Hahn, Hartley, and Rhoades have shown that the outgrowths which they studied on roots of conifers are of this nature.

In several plants, intumescences have been found which consist of enlarged cells which have been increased in number as well. Sorauer, Von Schrenk, Douglas, Wallace, and Dale have described abnormalities of this nature. Apparently the type of growth is determined by the nature of the stimulus which causes it, rather than by the species concerned; for Wolf found hypertrophies on cabbage leaves caused by wind-blown sand, while Harvey (5) found that freezing produced hyperplasias on leaves of the same plant.

The hypertrophies on *Populus grandidentata* and *P. tremuloides* consist of cells which have become greatly enlarged so that they push out from the leaf and rupture the epidermis. There is no evidence of a solution of cell walls such as Wallace (16) has described, nor do the cells ever become freed from each other or from the cells in the interior of the leaf. It is obvious that the growths on poplar leaves are very different from those on apple twigs described by Wallace. Externally the two types of growth are not much alike, and can be classed together only on the ground that both are small eruptions. However, it is probably not well at present to give new names to the two types into which one can readily divide the intumescences thus far described. The swelling of the cells described by Wallace has been found in a more exaggerated form on poplar leaves. Another feature common to the intumescences on apple twigs and those on poplar leaves is the development of two large vacuoles in many of the cells which gives an appearance of cell division.

Little is said about the rate of development of intumescences in the studies which have been made. Küster tells of their development on shells of peas within 24 hours, which is probably the most rapid growth recorded for any of these eruptions. Wallace found that in the apple responses might take place within 48 hours, or might be delayed for as long as six days. On leaves of *Populus grandidentata* and *P. tremuloides* a minimum time of 72 hours is needed for the outgrowths to become apparent, but many of them may not be initiated until a day or two later.

The difficulties which prevent the discovery of the fate of intumescences on poplar leaves, and which are inseparably correlated with the conditions of their development, have been discussed. Wallace and Dale have found that the growth of a layer of periderm may cut off the intumescences for normal tissues of the plants which they studied, but no evidence of periderm formation has been found in poplar leaves.

A discussion of the stimuli which initiate intumescences in the various plants on which they occur is reserved for a later paper.

SUMMARY

1. This paper reports the results of a study of the structure and development of intumescences on leaves of *Populus grandidentata* and *P. tremuloides.*

2. The intumescences develop rapidly on leaves confined in damp chambers, but are found in nature only on leaves which have been rolled or fastened together in pairs by insects.

3. *Populus grandidentata* and *P. tremuloides* are the only species of the genus which this study has shown to be capable of producing intumescences. *Populus deltoides, P. balsamifera, P. alba, P. simonii,* and *P. nigra* var. *italica* never produced intumescences under any of the conditions to which they were subjected.

4. Leaves of all ages, except those just ready to fall and those which have just emerged from the bud, are capable of developing intumescences.

5. The outgrowths occur on both laminae and petioles. On the laminae they appear on both upper and lower surfaces, but are most abundant on the lower surface. On either surface they are most abundant along the midribs and the larger veins.

6. The outgrowths are due to swelling of the cells, which are not increased in number. Therefore the abnormalities are to be considered hypertrophies.

7. The hypertrophied cells do not separate from one another, and no dissolution of any layers of the cell walls has been observed.

8. The leaves must be kept in damp chambers for three days before the outgrowths become apparent.

9. Under the conditions of these experiments, no periderm develops between the intumescences and the normal tissue of the leaves.

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SEASONAL AND DIURNAL VARIATIONS IN THE OSMOTIC VALUES AND SUCTION TENSION VALUES IN THE AERIAL PORTIONS OF *AMBROSIA TRIFIDA*[1]

ERVIN M. HERRICK

INTRODUCTION

The objects of this investigation were to determine the direction and magnitude of the gradients in osmotic values and suction tension values in the aerial portions of *Ambrosia trifida* L. and to determine the magnitude of the daily and seasonal variations in these values. *Ambrosia trifida*, commonly known as the giant ragweed or horseweed, was selected for this investigation because the necessary quantity of plant material of uniform quality was available at a relatively short distance from the laboratory.

The theory of the "Pull of Transpiration" (6, 11, 12) has been quite generally accepted as the best explanation of the mechanism by which water ascends to the aerial parts of plants. It is not the aim of this paper to discuss or question this theory. To accept this theory it is not necessary to assume, or even expect, that the highest portions of the plant will have the greatest "lifting power." However, each portion of the plant must be able to raise sufficient water to its own height and must be able to compete for water with other portions of the same plant, particularly when the water supply is deficient.

It might be assumed that the tissues capable of exerting the greatest tensions on the water columns will tend to be the most successful in the competition for water. As yet few data are available concerning the relative osmotic and suction tension values in the various portions of the same plant. Data are lacking particularly from the standpoint of the effect of a deficiency of water on these relative values.

Dixon and Atkins (9) studied the seasonal variations in osmotic value of the expressed sap of the leaves of several species of evergreen shrubs. In general, they found these to have a higher osmotic value in winter than in summer. Similar results on other evergreens were obtained by Lewis and Tuttle (22), Korstian (20), Gail (15), and others. Meyer (25) has pointed out that the rise in osmotic values in the leaves of evergreens in the winter is largely due to an increase in soluble sugars.

Several attempts have been made to determine whether or not osmotic value gradients exist from the root to the apical portions of the plant. Dixon and Atkins (10) showed that the concentration of sugars in the sap of the

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conducting tracts increases from lower to higher levels in the stem.   Harris, Gortner and Lawrence (18), in studying the osmotic value of tree leaves, reported higher osmotic values in the mature upper leaves of a tree than in the mature lower leaves of the same tree.   Ursprung and Blum, however, found that in leaves of the same age no satisfactory correlation between insertion height and osmotic value could be detected (33).   Ursprung and Blum have further pointed out that the highest osmotic values were sometimes found in the apical portions of the plant and sometimes below the apex (34).   Fernald (13), using 20 cm. portions of the stem of *Philadelphus*, showed that the expressed sap of the apical 20 cm. portion had a higher osmotic value than the expressed sap of any of the successive 20 cm. portions below the tip.   More recently Fernald (14), using 2 cm. portions of young shoots of *Asparagus*, has pointed out that the tissues having the greatest freezing point depression lie not at the tip but at a distance of 1 to 3 cm. from the tip.   In this later work Fernald worked with pieces of living tissue and determined the depression of the freezing point by the thermocouple method.

The pressure under which water tends to enter a living cell is physically equal to the osmotic value of the cell contents expressed in pressure units less the pressure exerted by the cell walls on the cell contents.   In vacuolated cells the maximum possible value of this pressure difference would be essentially equal to the osmotic value of the cell contents.   As water enters a plant cell the result is a gradual increase in turgidity of the cell, hence an increase in the pressure exerted by the cell walls on the cell contents.   This results in a diminution in the pressure difference and hence in the pressure with which water enters the cell.   When the cell becomes completely turgid the value of this pressure difference falls to zero.   At this point the cell is in dynamic equilibrium with its surrounding medium, *i.e.* water is entering and leaving the cell at the same rate but there is no net gain of water by the cell.   This pressure difference which determines the pressure with which water enters the cell has been variously named by different investigators.   Ursprung and Blum (36), Blum (3), Molz (27), and others use the term " suction force " and discuss in some detail what is meant by the term.   Thoday (31) used the term " water absorbing power " in preference to the term " suction force " as suggested by Ursprung and Blum.   Stiles (30), after consideration of other terms in use, preferred the term " suction pressure."   More recently Beck (1) proposed the term " suction tension " as being a more desirable one for this value.   Irrespective of the relative merits of these various terms it should be made clear that all of these have essentially the same meaning.   While none of the terms is entirely satisfactory the term " suction tension " or " suction tension value " will be used in this paper because it is probably the least objectionable of the various terms proposed up to date.   For the purpose of this paper suction tension will be defined as the initial pressure with which water will enter a living cell if that cell be placed in distilled water.   This pressure should be thought of as a definite physical factor.   It should also be

clear that a cell will have an actual suction tension value whether or not that cell is in water or in a position to take up water.

In 1916 Ursprung and Blum (35) described their first method of measuring suction tension of living cells, and made a study of the suction tension values in the leaves at various positions on the same plant (36). In general they found that the suction tension values were greater in the leaves at the tip of a branch two meters long than in the leaves at the base of the same branch and that there was a distinct increase in suction tension with an increase in height of leaf insertion. At a somewhat later date Ursprung (32) reported that leaves and flowers of *Bellis* showed a daily variation in suction tension values, reaching maximum values sometime after noon. He further reported a seasonal variation in suction tension values for leaves (entire year) and for flowers (June to November) of *Bellis,* maximum values being found in midsummer and in midwinter.

Molz (27, 28) has made a study of the suction tension values in a variety of plants in a wide range of habitats. He presents, however, no data on comparative suction tension values in the different parts of the same plant at any one time. Molz pointed out that suction tension values across the mesophyll of a leaf tend to show more consistent gradients than are shown by the osmotic values at incipient plasmolysis.

A study of osmotic values, measured by the plasmolytic method, has been made by Beck (1, 2) in which he has pointed out a lack of a consistent gradient in the osmotic values across the mesophyll of a leaf. He has, however, recognized the existence of suction tension gradients " in the direction of the streaming water." Beck has also shown that daily variations occur in the osmotic values within the leaf.

METHODS

In this investigation osmotic values were determined by the cryoscopic method in a manner similar to that described by Meyer (24), an electrically refrigerated low-temperature bath being used instead of the ice-salt baths described by Meyer. In this investigation all samples for cryoscopic determinations were frozen at $-30°$ C. before pressing out the sap. The necessity of treating such samples before expressing the sap has been pointed out by Dixon and Atkins (7, 8), Meyer (26), and others. In practice the samples, unless otherwise stated, were collected between 1:00 and 3:00 P.M. and immediately placed in a portable freezing bath at a temperature of $-15°$ to $-20°$ C. On returning to the laboratory the samples were transferred to a $-30°$ C. bath where they remained for 1 to 2 hours and, after rapid thawing, the sap was pressed out at a pressure of approximately 700 kg. per sq. cm. (10,000 pounds per sq. in.) (19). The sap samples were then stored at $-30°$ C. until the cryoscopic determinations could be made. In this investigation all samples for cryoscopic work were composite samples, each sample consisting of corresponding parts of 20 or more similar plants; hence the

values determined represent the average osmotic values for corresponding parts of 20 or more individual plants.

By keeping the tissue or sap samples constantly frozen, except while pressing out the sap, very little opportunity is afforded for chemical changes to occur that might alter the osmotic value of the frozen tissue or of the expressed sap. It has been necessary to assume that any changes that occur in the tissues, while frozen at these low temperatures, are insignificant and that in any one series of samples such slight changes as might occur would be of comparable magnitude and direction. Samples of sap immediately after being expressed from the thawed tissues were found to show the same freezing point depression as similar sap samples frozen at − 30° C. and again thawed before determining the freezing point depression. This method, however, does not preclude the possibility of osmotic changes occurring when the tissues are first frozen. The work of Carrick (4, 5) suggests that there might be a considerable change in osmotic value at that time.

The observed freezing point depressions have been corrected by the factors given in the table by Harris (16), this table being based on the formula of Harris and Gortner; $\Delta = \Delta' - 0.0125 \, u\Delta'$ (17). From the corrected freezing point depressions, the osmotic values have been obtained from the table by Harris and Gortner (17), this table being based on the equation O.P. $= 12.06\Delta - 0.021 \Delta^2$ (21). In this paper all osmotic values are given in terms of atmospheres as at 0° C.

In the investigation of suction tension values the method used was a modification of the " simplified method " introduced by Ursprung and Blum (35, 36, 37). Owing to the fact that numerous minor details of their technique have been altered or omitted a brief description of the method used will be given. The standard procedure employed in the study of suction tension was to cut strips of the leaves 30 mm. × 4 mm. and determine their length at once, in the field, on a measured slide. The measured slide used in this work was a glass slide with two parallel lines cut across the surface about 30 mm. apart. The strip of leaf tissue to be measured was placed on the slide and its length measured as being plus or minus from the two cross lines on the slide. For this measurement a microscope equipped with a 32 mm. objective and a 12.5 × ocular, fitted with an ocular micrometer, was used. The measured strip was then placed in a one-dram (3.7 cc.) vial containing about 2 cc. of sucrose solution of known osmotic value. A series of strips were measured in this manner and placed in a series of sucrose solutions. After one and a half hours the strips were again measured and the solution that had produced no change in length of the strip was considered to be in dynamic equilibrium with the tissue, hence a measure of the suction tension value (35, 37). Preliminary tests showed that one and a half hours was long enough to allow between the first and final measurements in determining suction tension values. The osmotic value of the solution that had produced no change in length of the tissue, as determined by the cryoscopic method, was considered to be equal to

the average suction tension value of the cells of the tissue as at 0° C. This method of measuring can be considered reliable only as long as the tissues are practically or completely impermeable to sucrose under the above conditions.

In this investigation fresh sucrose solutions were made up each day to avoid the use of toluene or other preservatives, the effects of which on permeability may not be fully understood. The solutions were made up at intervals of 0.04 weight molar sucrose, preliminary determinations having shown that such intervals correspond to intervals of approximately one atmosphere at 0° C. These preliminary tests showed the osmotic value of a weight molar sucrose solution to be 25.7 atmospheres at 0° C. Morse (29) gives the osmotic value of a weight molar sucrose solution to be 24.826 atmospheres at 0° C. A similar close agreement was found between these preliminary data and the tables by Morse for all concentrations between 0.2 and 1.0 weight molar sucrose solutions. The differences between the values obtained in this preliminary work and the values given by Morse are probably due principally to differences in the quality of sucrose used. Morse used specially purified sucrose while in this investigation commercial sucrose was used. While commercial sucrose is a highly purified product, traces of glucose and fructose present might appreciably affect the osmotic values of its solutions. The values obtained with commercial sucrose have been used in preference to the values given by Morse as such values were considered to be more nearly correct for the solutions used in this investigation than are the values given by Morse.

Throughout this work portions of the various sucrose solutions were discarded after having been used once. This was considered to be necessary to avoid the introduction of errors in subsequent determinations as a result of changes in osmotic values due to the immersion of the tissue strips in the solutions. Molz (27) considers it to be permissable to use sucrose solutions as often as four times, however, he makes no statement as to the relative amounts of solution and of tissue used. The amount of change in osmotic value of such solutions would depend on the relative amount of tissue and of solution used, as well as on certain other factors such as the difference between the suction tension value of the tissue and the osmotic value of the solution being used.

In this investigation dry weight determinations were made by drying the appropriate samples *in vacuo* for 72 hours at 72° C. Preliminary work with the oven used showed that constant weight was obtained by such procedure. Throughout this paper the loss of weight *in vacuo* at 72° C. for 72 hours will be regarded as water content, the values for water content being expressed as percent of fresh weight.

The experimental work of this investigation was carried on during the summer of 1931 at Columbus, Ohio. The necessary plant material was collected on the flood plains of the Olentangy river, adjacent to the Ohio State University campus, where an abundance of *Ambrosia trifida* was available.

To follow the seasonal changes in osmotic values, the osmotic value gradients through the various leaves and internodes were determined at intervals of approximately one week from the seedling stage (May 2d) to midsummer (July 15th). During this period it was possible to follow the plants from the seedling stage almost to maturity, this period representing a seasonal change from cool spring weather to a condition of extreme heat and drought in midsummer.

<div align="center">RESULTS AND DISCUSSION</div>

Table 1 summarizes the seasonal variations in osmotic values found in the aerial portions of *Ambrosia trifida*. Table 2 summarizes the seasonal variations in water content of the aerial portions of this species.

Ambrosia trifida, under conditions of decreasing water supply and increasing soil and air temperatures, shows a definite increase in osmotic values throughout the plant. This increase in osmotic values became especially apparent during the period between June 24th and July 15th, during which time the soil became very deficient in moisture and hot, clear weather prevailed. The slight drop in osmotic values from July 8th to 15th can be satisfactorily accounted for only as an effect of prolonged wilting on photosynthesis, as there was no rain during the intervening week and no cool or cloudy weather. The plants were wilted practically throughout all the daylight hours of this week of rather extreme drought. A similar rise and fall in osmotic values was observed in case of *Helianthus annuus* L. grown in the greenhouse under artificial drought conditions, the rise and fall being even more pronounced than in *Ambrosia trifida*.

The osmotic values of the several internodes of the stem usually showed a consistent gradient of increasing values from the hypocotyl to the highest internode, as shown in table 1. The osmotic values of the leaves usually failed to show a consistently increasing gradient from lower to higher leaves. This lack of gradient in the leaves will be more fully discussed in connection with tables 3 and 4.

Weekly determinations of the osmotic values of the internodes show a gradual decrease in osmotic value with increasing age of the internode during the period from May 2d to June 24th. This decrease in osmotic values was interrupted by a definite rise in osmotic values with increasing drought conditions from June 24th to July 15th. Under conditions of sufficient water a rise in osmotic values in these portions could be accounted for only as being due to an increase in soluble carbohydrates. Under drought conditions, however, this marked increase in osmotic values must be due not only to an accumulation of soluble carbohydrates, but, to some extent at least, to a loss of water. From the data available at the present time it is impossible to determine the relative importance of these two factors.

An examination of the vertical columns of osmotic values for leaves, as given in table 1, shows that the osmotic value of the topmost leaf is sometimes, but not always, greater than the osmotic value of the next lower leaf.

TABLE 1. *Seasonal Variation in the Osmotic Values of Ambrosia trifida*

Date	5/2	5/8	5/14	5/19	5/27	6/3	6/9	6/18	6/24	7/1	7/8	7/15*
9th leaf	—	—	—	—	—	—	—	—	—	13.4	17.4	17.1
8th leaf	—	—	—	—	—	—	—	—	11.6	13.5	17.2	15.9
7th leaf	—	—	—	—	—	—	—	9.9	11.5	11.4	17.1	15.8
6th leaf	—	—	—	—	—	—	10.1	10.2	10.9	—	15.9	15.1
5th leaf	—	—	—	—	—	10.1	11.8	11.1	9.3	—	—	—
4th leaf	—	—	—	—	—	8.8	10.5	9.5	7.3	—	—	—
3d leaf	—	12.3	8.6	8.4	9.9	8.4	6.6	—	—	—	—	—
2d leaf	9.8	14.1	10.2	10.6	9.6	8.5	—	—	—	—	—	—
1st leaf	9.5	8.7	7.1	8.7	—	—	—	—	—	—	—	—
Cotyledons	7.4	6.4	—	—	—	—	—	—	—	—	—	—
8th internode	—	—	—	—	—	—	—	—	—	12.6	16.1	15.6
7th internode	—	—	—	—	—	—	—	—	11.0	12.7	16.0	15.1
6th internode	—	—	—	—	—	—	—	10.9	10.9	10.2	15.2	15.0
5th internode	—	—	—	—	—	—	9.6	9.9	8.5	10.2	14.4	14.6
4th internode	—	—	—	—	—	10.0	10.0	7.5	7.5	8.6	13.2	14.6
3d internode	—	—	—	8.7	9.7	9.5	8.8	7.1	7.5	8.4	12.3	13.8
2d internode	—	9.6	9.2	8.4	9.8	9.0	7.1	6.9	—	8.2	11.7	12.7
1st internode	10.2	10.2	9.5	7.8	8.9	8.9	—	—	—	—	11.6	12.5
Hypocotyl	9.3	9.5	8.7	7.8	8.2	—	—	—	—	—	—	—
Soil water	—	—	26%	23%	27%	20%	22%	19%	12.6%	10.3%	7.6%	—
Air Temp. °C	—	—	—	29° C.	32° C.	34° C.	28° C.	29° C.	33° C.	40° C.	34° C.	38° C.

*At this time the plants were approximately 2 meters in height.

TABLE 2. *Seasonal Variation in the Water Content of Ambrosia trifida*

Date	5/2	5/8	5/14	5/19	5/27	6/3	6/9	6/18	6/24	7/1	7/8	7/15*
9th leaf	—	—	—	—	—	—	—	—	—	75.0	65.2	66.1
8th leaf	—	—	—	—	—	—	—	74.2	75.5	70.5	66.2	66.2
7th leaf	—	—	—	—	—	—	—	74.3	75.1	69.8	65.4	66.4
6th leaf	—	—	—	—	—	—	—	76.6	74.1	—	71.4	65.7
5th leaf	—	—	—	—	—	—	—	83.0	76.5	—	—	—
4th leaf	—	—	—	—	—	76.3	—	—	81.8	—	—	—
3d leaf	—	—	—	—	79.8	78.2	—	—	—	—	—	—
2d leaf	78.5	—	82.1	79.1	—	78.5	—	—	—	—	—	—
1st leaf	81.2	85.7	82.3	80.8	81.3	82.3	—	—	—	—	—	—
Cotyledons	90.2	84.0	84.9	85.1	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—
8th internode	—	—	—	—	—	—	—	—	—	—	—	80.6
7th internode	—	—	—	—	—	—	—	—	—	—	83.1	79.3
6th internode	—	—	—	—	—	—	—	—	—	83.6	83.4	75.6
5th internode	—	—	—	—	—	—	—	87.1	85.5	83.2	82.9	75.1
4th internode	—	—	—	—	90.7	90.5	—	87.1	86.1	83.0	81.7	74.9
3d internode	—	—	—	91.8	90.7	89.6	—	87.5	87.2	82.7	80.8	77.5
2d internode	—	90.1	94.2	91.2	88.4	87.1	—	87.6	87.2	82.6	79.7	77.0
1st internode	88.1	87.3	88.7	86.0	87.4	86.0	—	87.0	88.4	82.7	78.4	77.3
Hypocotyl	85.6	81.8	83.1	83.5	84.9	85.0	—	84.2	88.1	—	77.5	—

* At this time the plants were approximately 2 meters in height.

An examination of the horizontal rows of osmotic values, however, usually shows an increase in osmotic values for corresponding leaves between the immature stage when first measured and the more mature stage a week later. This increase in osmotic values with increasing age of the leaf was followed by a decrease in osmotic values with further increase in age. This period of decreasing osmotic values corresponds to the period during which the leaves at any one position were increasingly shaded by further development of leaves and stem above that position. With increased development of the leaves and stem above, the leaves at any one position ultimately turn yellow and die. During the drought period from June 24th to July 15th a steady increase in the osmotic values was found in all the leaves as well as in all the internodes of the stem. An examination of any one series of determinations, however, shows the lowest leaf invariably to have a lower osmotic value than the other leaves. From table 1 it is evident that the osmotic value of the apical portion of the stem is similar to that of the top or next to top leaf. The osmotic value of the upper leaves was usually slightly higher than that of the apical portion of the stem; the difference, however, was usually not over one or one and a half atmospheres.

During the eleven weeks of this investigation the water content of all the aerial parts of *Ambrosia trifida* showed a definite decrease, as shown in table 2. The water content showed a slight tendency to increase between July 8th and 15th but this increase was probably due to the same factors that decreased the osmotic values in the same period, *i.e.*, this apparent increase in water may represent only a loss of soluble sugars, rather than an absolute increase in water content.

In order to study in more detail the changes in osmotic value and water content under conditions of "stress" or internal competition for water, a series of determinations were made at intervals of 3 to 4 hours from sunrise to sunset. The data presented in tables 3 and 4, below, are for July 8th, 1931. Owing to a slight dew the early morning samples were not collected until 6:00 A.M., this being approximately 45 minutes after sunrise. The late afternoon samples were collected at 8:00 P.M., this being within a few minutes of sunset. Table 3 summarizes the daily variations in osmotic values as found in the aerial portions of *Ambrosia trifida* under conditions of high temperature and low soil water content. Table 4 summarizes the daily variation in water content of the aerial portions of *Ambrosia trifida* as found under the above conditions.

Table 3 suggests an explanation of the usual lack of a consistently increasing gradient in osmotic values from the lower to higher portions of the same plant, as shown in table 1. The type of gradient is seen to depend on external factors and daily variations in these factors are reflected in the type of gradient shown by the osmotic values. At 6:00 A.M. all of the leaves were turgid and the highest osmotic value was found in the second leaf from the top. By 10:00 A.M. all but the top leaf were slightly wilted and the greatest os-

motic value was found in the top leaf. The top leaf was the only one to
remain erect until sunset, the highest osmotic value being found in the top
leaf throughout the afternoon. The maximum osmotic values in all parts of
the plant were reached at about 2 :00 P.M.

A comparison of tables 3 and 4 shows a definite decrease in water content
as the osmotic values increase, followed by an increase in water content as
the osmotic values decrease in the afternoon. This, however, does not tell
whether the rise in osmotic value and the fall in water content are due to a

TABLE 3. *Diurnal Variations in Osmotic Value in Ambrosia trifida*

Part Used	6 A.M.	10 A.M.	2 P.M.	5 P.M.	8 P.M.
Top leaf.....	12.52	12.59	17.23	16.47	16.33
Next to top leaf	12.52	15.63	17.05	15.65	14.90
2d from top leaf	12.90	15.04	16.98	15.65	14.90
Lowest leaf..........	10.10	13.00	15.76	14.26	14.03
8th internode	12.74	15.28	16.01	14.63	15.02
7th internode.	11.68	14.72	15.96	14.38	14.27
6th internode	11.19	13.84	15.14	13.29	12.66
5th internode.	10.92	13.42	14.46	12.04	11.99
4th internode.	9.48	12.49	13.18	11.84	10.98
3d internode.	9.37	11.25	12.24	11.33	10.66
1st internode	8.84	10.44	11.46	9.86	8.93

TABLE 4. *Diurnal Variations in Water Content in Ambrosia trifida*

Part Used	6 A.M.	10 A.M.	2 P.M.	5 P.M.	8 P.M.
Top leaf......	72.4%	68.0%	66.1%	67.4%	68.6%
Next to top leaf	74.1	66.3	66.2	67.0	67.5
2d from top leaf	74.2	66.7	66.4	66.7	67.4
Lowest leaf.....................	68.6	66.6	65.7	66.2	67.3
8th internode..................	83.3	80.6	80.6	81.1	81.5
7th internode	80.7	79.7	79.3	80.7	80.9
6th internode	77.2	76.9	75.6	77.6	78.0
5th internode	76.9	76.2	75.0	76.1	76.8
4th internode	77.8	76.3	74.9	75.4	76.7
3d internode	78.3	78.2	75.7	76.1	76.3
1st internode	79.5	79.4	77.3	77.6	79.2
Air temperature	23° C.	38° C.	38° C.	38° C.	34° C.

loss of water, an increase in soluble carbohydrates, or both. It is evident
that the decrease in water content is one of the factors involved in the in-
crease in osmotic values.

Text figures 1 and 2 summarize the changes in osmotic values during a
change from conditions of adequate water supply to conditions of severe in-
ternal stress due to a deficiency of water. Figure 1 compares the values in
different parts of similar plants at 6:00 A.M. and 2:00 P.M. on a cloudless
day in midsummer (July 8th). On this day the temperature at 6:00 A.M.
was 23° C. and at 2:00 P.M. 38° C. Figure 2 compares values in different
parts of similar plants on June 18th and July 8th at the same hour of the

3

day. June 18th was also a cloudless day and was relatively cool as compared with July 8th. The temperature at 2:00 P.M. on June 18th was 28.5° C., on July 8th the temperature at 2:00 P.M. was 38° C.

From table 3 and text figures 1 and 2, it is apparent that the osmotic value gradient may vary at different times of the day. It is evident that under conditions of sufficient water supply the greatest osmotic value is not found in the youngest or top leaf. As the stress for water becomes greater the osmotic values increase throughout the plant, the upper leaves showing relatively the greatest increase. Under conditions of severe stress the highest osmotic value is found in the top leaf and remains there as long as the con-
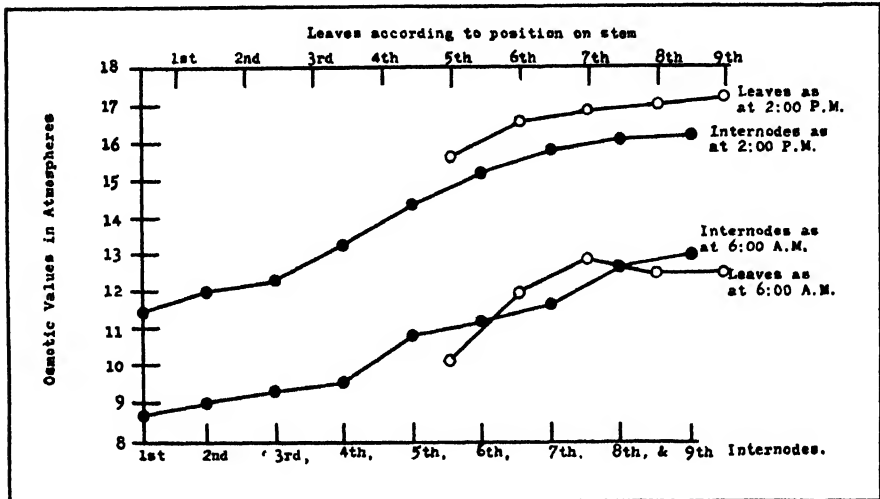
TEXT FIG. 1. A comparison of the relative osmotic values in the aerial parts of *Ambrosia trifida* under conditions of severe stress at 2:00 P.M. and not under severe stress at 6:00 A.M. Data are for July 8, 1931. The leaves are numbered according to their sequence of development, 1st leaf above the cotyledons, 2d leaf above the cotyledons, etc. The internodes of the stem are numbered in order of their position above the hypocotyl, 1st internode being between the cotyledons and the first true leaves, 2d internode between the first and second pairs of true leaves, etc.

ditions of stress continue. Under such conditions the osmotic values show a gradient of consistently increasing osmotic values from the lowest to the highest leaf.

During a period of drought the plants may have sufficient water in the early morning hours but 6 to 8 hours later the competition for water may be very severe. Under such conditions the osmotic values increase rapidly throughout the plant and the osmotic gradient rapidly becomes steeper. Text figure 1 shows the rapid change in relative and absolute values in the various aerial parts of *Ambrosia trifida*. A strikingly similar change in osmotic values was found when data for June 18th were compared with data for July 8th at the same hour of the day, as shown in text figure 2. This seasonal change was correlated with a change from conditions of ade-

quate water supply to conditions of severe drought three weeks later, the soil water content falling from 19 percent to about 8 percent. A comparison of the two figures shows that the changes in osmotic values were approximately the same whether the stress for water was brought about by seasonal or diurnal changes.

The relatively large increase in osmotic values under conditions of increasing stress for water suggests that an absolute decrease in water content is an important factor in this increase in osmotic values. An absolute increase in soluble sugars would also increase the osmotic values and it seems
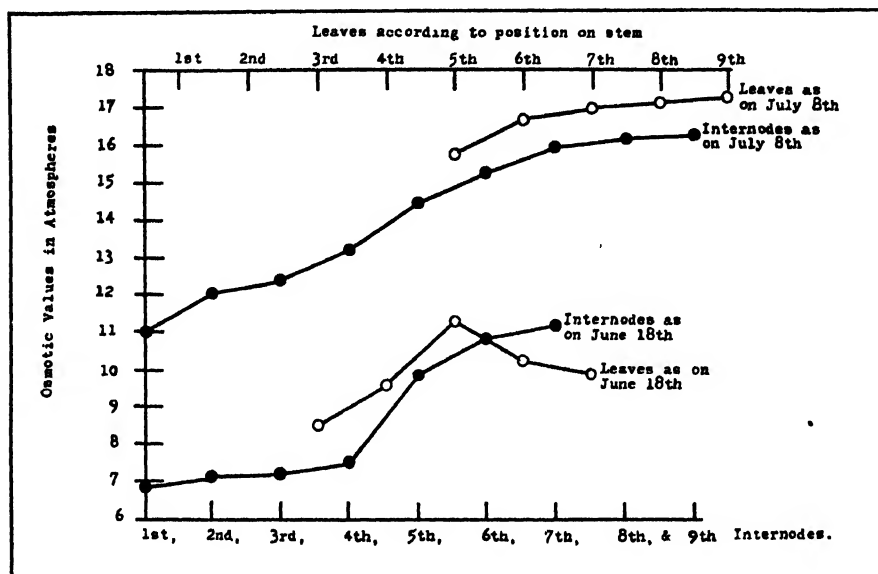
TEXT FIG. 2. A comparison of the relative osmotic values in the aerial parts of *Ambrosia trifida* under conditions of severe stress on July 8th and not under severe stress on June 18th. Data are given for 2:00 P.M. The leaves are numbered according to their sequence of development, 1st leaf above the cotyledons, 2d leaf above the cotyledons, etc. The internodes of the stem are numbered in order of their position above the hypocotyl, 1st internode being between the cotyledons and the first true leaves, 2d internode between the first and second pairs of true leaves, etc.

probable that both factors are involved in this increase, whether seasonal or diurnal.

In addition to the above study of osmotic values in the various portions of the plant, a comparison was made of the daily variation in osmotic and suction tension values of the leaves at various heights on the plant ranging from the lowest living leaf of the plant to the very immature leaves at the apex. In this work no attempt was made to correlate fluctuations of suction tension values with any one environmental factor. For a discussion of the effect of the various environmental factors on suction tension values the works of Blum (3), Molz (27, 28), and Li (23) should be consulted.

The suction tension values of the leaves of numerous individual plants of *Ambrosia trifida* were determined and these values were found to vary widely among apparently similar plants of the same species in the same habitat. On any one plant, however, opposite leaves of the same pair ordinarily showed the same suction tension values.

To compare the osmotic and suction tension values, a number of series of suction tension determinations were made on four similar, consecutive, hot days. These values were averaged and compared with a series of osmotic determinations made at 3 to 4 hour intervals on the fourth day, July 8th. The comparative values are summarized in table 5. The suction tension and osmotic values are both stated as atmospheres as at 0° C.

TABLE 5. *Diurnal Variation in Osmotic Values and Suction Tension Values in Leaves of Ambrosia trifida*

Time	Top Leaf	Next to Top	2d from Top	Lowest Leaf	Temp.
A. Osmotic Values (atmospheres)					
6:00 A.M.	12.5	12.5	12.9	10.1	23° C.
10:00 A.M.	15.3	15.6	15.0	13.0	38° C.
2:00 P.M.	17.4	17.2	17.1	15.9	38° C.
5:00 P.M.	16.5	15.7	15.7	14.3	38° C.
8:00 (Sunset)	16.3	14.9	14.9	14.0	34° C.
B. Suction Tension (atmospheres)					
5:15 (Sunrise)	7.5	8.0	8.0	6.5	23° C.
7:00 A.M.	9.5	9.5	8.5	7.5	27° C.
9:00 A.M.	12.0	12.0	11.0	11.0	33° C.
11:00 A.M.	15.0	15.0	14.5	13.5	38° C.
1:00 P.M.	17.5	17.0	16.5	14.5	39° C.
3:00 P.M.	17.5	17.5	17.0	15.5	38° C.
4:30 P.M.	15.0	16.0	15.5	14.5	38° C.
7:30 P.M.	15.0	15.0	15.0	14.0	35° C.

An examination of the above table shows that both the osmotic and suction tension values increase to a maximum at about 2:00 P.M., and decrease from that time until sometime after sunset. In all the leaves for which determinations were made the maximum osmotic and suction tension values were found at about the same time.

At 6:00 A.M. the osmotic values were distinctly higher than the suction tension values for 5:15 or 7:00 A.M. At that time neither the osmotic values nor the suction tension values showed a maximum in the top leaf. By 10:00 A.M. both the osmotic values and suction tension values had increased but the osmotic values were still distinctly higher than the suction tension values. The maximum osmotic value was found in the next to the top leaf but the suction tension values of the top and next to top leaf were equal, both being higher than in the lower leaves. By 2:00 P.M. the osmotic values had reached their highest values in all the leaves, the maximum being found in the top leaf. By 1:00 P.M. the suction tension values showed a consistently increasing gradient from the lowest to the highest leaf, the maxi-

mum being in the top leaf. By 3:00 P.M., however, the next to top leaf was found to equal the top leaf in suction tension value. By 5:00 P.M. the osmotic values still showed a consistent gradient with the maximum in the top leaf but the values were somewhat lower than at 2:00 P.M. By 4:30 P.M. the suction tension values no longer showed a consistent gradient from the lower to the upper leaves and the values were lower than at 3:00 P.M. By 8:00 P.M. the osmotic values still showed a distinct maximum in the top leaf. At that time the suction tension values showed essentially no gradient, being nearly equal in all the leaves, i.e., the stresses within the plant were equilibrated. Presumably this condition would be maintained until the early morning hours of the following day.

Under conditions of maximum suction tension and maximum osmotic values (maximum competition for water) both the osmotic and suction tension values show consistently increasing gradients from the lowest to the highest leaves on the plant. Under conditions of increasing or maximum stress for water the maximum osmotic and suction tension values are found in the immature upper leaves of the plant. Under such conditions the suction tension value of any one leaf is essentially equal to the osmotic value of that same leaf.

It has been frequently observed that under conditions of drought the lower leaves of *Ambrosia trifida* wilt first and the immature upper leaves are the last to wilt. This order of wilting of the various leaves appears to be inversely the order of their relative potential ability to secure water under conditions of stress, as determined by a measure of the osmotic or suction tension values. Since the osmotic and suction tension values become essentially equal under conditions of stress, and since the osmotic value represents the maximum suction tension value for vacuolated cells, it is apparent that a measure of the osmotic values serves as a reliable index of the relative potential ability of the various aerial parts of a plant to compete for water under conditions of stress. Such measurements would, of course, be of greatest significance if they were made while the plant was under actual conditions of internal stress for water.

SUMMARY

A study has been made of the osmotic and suction tension values in the aerial portions of *Ambrosia trifida* L.

Osmotic values were determined by the cryoscopic method, using expressed saps. Suction tension values were determined by a modification of the so called " simplified method " of Ursprung and Blum. Water content was determined by drying *in vacuo* for 72 hours at 72° C.

The several internodes of the stem usually showed a gradient of consistently increasing osmotic values from the hypocotyl to the immature internodes near the apex of the stem. Under conditions of adequate water supply no consistent gradients in osmotic values were found in the leaves of

this plant. The osmotic values of the leaves are usually higher than the osmotic values of the adjacent internodes of the stem. Increasing drought conditions resulted in increasing osmotic values throughout the aerial portions of the plant.

As the leaves or internodes mature their osmotic values increase and after attaining a maximum gradually decrease as they grow older. This decline in osmotic values may be prevented or delayed by drought conditions.

When the competition for water is not severe neither the osmotic nor suction tension values reach a maximum in the top leaf or apical portion of the stem, nor is there a consistent gradient in osmotic or suction tension values throughout the plant. Increasing internal competition for water, whether due to seasonal or diurnal causes, results in an increase in the magnitude of the osmotic values throughout the plant and the establishment of consistently increasing gradients in the osmotic and suction tension values from the lower to higher portions of the plant. Under such conditions of stress the osmotic values of the leaves in all cases were found to be greater than the osmotic values of the adjacent internodes of the stem. Under conditions of severe stress the osmotic value of any one leaf is essentially equal to the suction tension value of that same leaf. Under these conditions of stress the osmotic and suction tension values are slightly higher in the immature upper leaves than in the apical portion of the stem. The changes in osmotic value brought about by increasing stress for water were found to be of about the same magnitude whether the conditions of " stress " were brought about by seasonal or diurnal changes of environment. Both the osmotic and suction tension values tended to reach maximum daily values between 1 :00 and 3 :00 P.M.

The order of wilting of the various leaves was observed to be inverse to the order of their relative potential ability to secure or retain sufficient water.

It is apparent that a measure of the osmotic values serves as a reliable index of the relative potential ability of the various aerial parts of a plant to compete for water under conditions of stress due to a deficiency of water. Such measurements are of greatest significance if the determinations are made at a time when the plant is actually under stress for water.

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THE ANATOMY OF THE EMBRYO OF *CEDRUS* IN THE DORMANT STAGE

J. T. Buchholz and Edna M. Old

There are some very interesting anatomical features which become differentiated in the embryos of matured conifer seeds. Some differences may be found between the embryos of different genera and families, even though the plan of meristematic organization in the embryo is remarkably uniform throughout the Coniferales. A survey of the literature shows that relatively little which has to do with details in cellular arrangement and organization has been recorded. Most of the investigations have been concerned with the gross external morphology including size, shape, and number of cotyledons.

Schleiden (13), in his text-book, was among the first who gave a description of the half-grown embryo of a pine and of the embryo in the stage of the matured seed. He reported the order of appearance of the primordia for the organs: root tip, stem tip, and cotyledons. Strasburger (14), the largest single contributor on this subject, corrected Schleiden's error concerning one feature, namely, that it is the plerome apex of the root and not the true root tip which is the first part of the embryo to become differentiated. Schacht (12) described the meristem of the root tip in a pine and Strasburger gave us more fully the meristematic organization of the root tip and stem tip of various conifers. He also gave a description of the more mature embryos of *Thuja occidentalis*, *Pinus pumilio*, *Picea vulgaris*, *Taxus baccata* and *Ephedra altissima*, pointing out the great uniformity in the anatomical structure and organization of the embryo in conifers. His investigations did not include *Cedrus* which has, at least externally, some striking differences from most of the remaining genera of Abietineae.

A few other stages of conifer embryos have occasionally been described such as the descriptions of germinating seedlings by Lord Avebury (11). In taxonomic monographs and general text-books there are scattered records concerning the size, shape and the number of cotyledons, and occasionally these show sections of seeds or the embryos removed from the seeds.

In an extensive series of studies of the vascular anatomy of conifer seedlings, Hill and De Fraine (7) have given a comprehensive comparison of the seedlings in stages after the early vascular elements are well organized. From these we know the facts concerning the number of cotyledons in a large series of species, with records of the vascular connections in the root and transition region, occurrence and prevalence of fused cotyledons and cotyledonary tubes, divided or fused vascular strands, etc.

Hutchinson (8) has represented by means of a diagrammatic figure the general features of the embryo of *Keteleeria Fortunei,* as found in the embryo of the dormant stage, or possibly after the beginning of seed-germination. This description shows a union of the four cotyledons into a remarkably developed cotyledonary tube, and the embryo has a peculiar structure resembling a broad, projecting root cap. Clare and Johnstone (6) have shown the external appearances of embryos dissected from the mature seeds of several large-seeded species of pine, including some interesting records of polyembryony. One of us has given the figures and brief description of half-grown and of mature embryos of *Cephalotaxus Fortunei* (3) and of *Sciadopitys verticillata* (5). The outstanding feature of *Cephalotaxus* is the delay in the organization of the stem-tip meristem. In *Sciadopitys* the stem tip primordium is also somewhat tardy in its appearance. *Pinus* (1), and as far as known all of the Abietineae (2), have their stem-tip meristem organized and distinctly recognizable before the primordia of the cotyledons appear.

Aside from these accounts, there is very little in the literature concerning the anatomy of the coniferous embryo, but that which is known indicates that there are some noteworthy differences aside from variations in the number of cotyledons. We have made examinations of the embryos of the seeds of a dozen or more genera of conifers, and have selected two species of *Cedrus* as the subject of this investigation, because these differ somewhat from the related genera described by Strasburger and seem to embody some of the features found in *Keteleeria.* Doubtless the dormant stage of the embryo affords a fairly comparable stage in which it may be desirable to make comparisons with the embryos of many other conifers.

MATERIAL AND METHODS

The embryos used in this investigation were dissected under a binocular microscope from ordinary commercial seeds which had been kept in distilled water for two days. This softening was necessary in order to prevent injury to the embryos during their removal from the endosperm. The embryos enlarged slightly through imbibition but showed no mitotic figures or other signs of active growth. When we discovered plumular leaf primordia in the embryos of *Cedrus libanotica,* the dry seeds of these were examined by dissection under higher magnification. These showed the same condition as in the sections of practically all of the commercial seeds, and we feel assured, therefore, that these structures were not the result of growth stimulated after they had been placed in water, but were actually present in the dry seeds. After this investigation had been practically completed, some seeds of *C. libanotica* were obtained from the large tree at Flushing, N. Y. These came from a single large cone of the crop matured in 1930 which yielded hundreds of sound seeds and was removed from the tree in July, 1931. These seeds were somewhat larger throughout than the commercial seeds, but since no

important difference could be noted upon external examination of about 40 dissected embryos, aside from smaller plumule primordia, these were not sectioned.

Formalin-acetic-alcohol was used as a fixative and the standard procedure was followed in imbedding the material, with chloroform as the paraffin solvent. Sections were cut serially and stained in Heidenhain's haematoxylin and counterstained with Orange II dissolved in clove oil in order to bring out the cell walls. The drawings for text figures 2 and 3 were made with micro-projection apparatus giving a magnification of 100. It seemed impossible to draw each cell in an entire embryo in the hope of having it show in a reduced reproduction at this magnification. Since it was desirable for our present purpose to represent the entire embryos in some way rather than the details in certain regions only, we decided to employ a method which gives the effect of shading, but which is accurate at the same time in respect to finer details. Each cell (figs. 2 and 3) was represented by a short line placed in the apparent long axis of the cell. The long cells in the plerome region were represented by proportionally longer lines placed closer together where the cells were narrow, and cells not distinctly elongated were represented by dots. These lines were inked with a ruling pen, heavy enough to reproduce when a drawing approximately a meter long was reduced to the length of the printed page. Where mucilage or resin canals were found, these were likewise indicated by still broader lines. While these figures give the appearance of shaded diagrams, they are accurate enough to indicate the approximate number of cells in length and in diameter, together with their orientation. They are accompanied by more detailed drawings which show the outlines of the cells for certain regions selected from text figure 2. The enlarged parts are indicated by letters in figures 4A, B, and C.

ANATOMICAL FEATURES OF CEDRUS

Text figure 1 represents an external view of the embryo of *Cedrus libanotica*, and figure 2 shows it in section; figure 3 is that of *C. atlantica*. The embryos of both of these species are usually more or less bent or slightly twisted within the seed, but we have modified our drawings of the sections to represent them straight. The proportions in width and length are correct for the cotyledons and other parts. It will be noted that in *C. libanotica* the primordia of the first plumular leaves have already appeared. This portion of figure 2 is shown in greater detail in figure 4.1. The stem tip is only slightly convex and is marked by cells somewhat larger than the neighboring cells.

Aside from the well developed primordium of a plumular leaf, there is a very young primordium of an inner circle of leaves. The beginning of a leaf primordium may be recognized by the appearance of periclinal walls in the superficial cells, followed by divisions in the underlying cells. As Strasburger (14) has pointed out, this indicates that a dermatogen is not fully differentiated in this region.
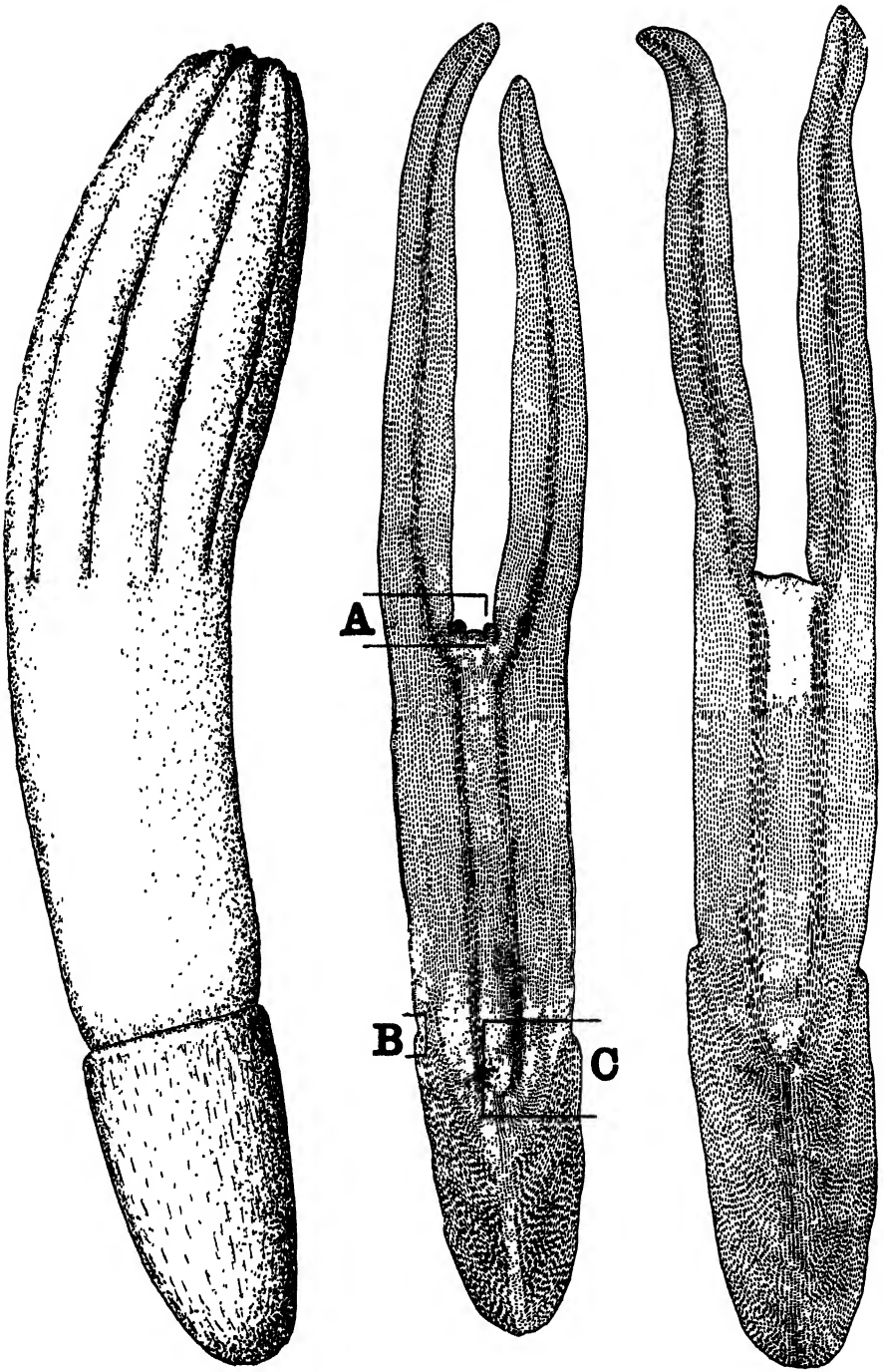
TEXT FIGS. 1-3. FIG. 1 (left). External appearance of embryo of *Cedrus libanotica* as dissected from mature seed. From seeds of tree at Flushing, N. Y. ×16. FIG. 2 (middle). Longitudinal section of an embryo of *C. libanotica,* showing arrangement of cells. Each line in the shading represents a cell. From commercial seeds. ×16. (Details at A, B, and C, shown enlarged in figure 4.) FIG. 3 (right). Longitudinal section of embryo of *C. atlantica.* ×16.

Our commercial seeds of *C. libanotica* all had embryos with an outer circle of plumular primordia well developed, and in some cases there were still younger primordia in a circle within these. In seeds collected from the large Cedar of Lebanon at Flushing, N. Y., there were no inner leaf primordia and in many of the embryos the outer circle could not be identified upon superficial examination with a 30 X binocular, even though these embryos were somewhat larger. Their relative stage of development at seed maturity falls between the stages shown by figures 2 and 3. It is probable, therefore, that the source of the seed may have something to do with their degree of development at the time of shedding. Probably the length of season and other climatic factors are responsible for these differences.

Figure 4*A* shows the procambium strands passing up into the base of a cotyledon and one of the elements is differentiated into a spiral element. Beginning at the point shown in figure 4*A* a row of spiral elements may be traced up through more than half of the length of the cotyledon. It appears that the protoxylem is endarch or very nearly so in the cotyledon. A similar condition was reported for *Ketelceria* (8). These earliest vascular elements were only found in *C. libanotica,* which were the embryos with the ring of plumular primordia, and Hutchinson's figure which records the spiral elements also has the primordia shown on the plumule. His figure shows ordinary spiral vessels, while *C. libanotica* has the earliest spiral elements interrupted by bordered pits as shown in figure 4*G*. Jeffrey (9) shows these in the primary wood of the roots and stems of a number of conifers (for example, figs. 117 and 156) without special comment, where they appear to be transitional between strictly spiral elements and tracheids. However, we have found no spiral elements without these pits in the cotyledons, where the earliest vascular structures become differentiated. No vascular elements were found in the embryo below this point, and no phloem elements could be recognized at this stage.

The epidermis of the lower portion of the cotyledon and the hypocotyl region is well differentiated and at places the hypodermal cells are shown giving rise to mucilage or resin canals. In this transformation the cells elongate greatly, their nuclei also enlarge many times, after which the cell contents stain much more deeply than the neighboring cells.

In the central or hypocotyl region of the embryo, all cells except the epidermal cells are elongated. The cortex is about 16 cells wide, the procambial part of the central plerome cylinder is a band of much narrower cells 7–10 cells wide, surrounding a central portion about 12 cells in diameter made up of larger cells. The plerome comes to an end below in a rounded dome. These details are shown in figure 4*C,* which is an enlarged portion of figure 2 at *C*.

Several large resin cells are indicated by the black streaks in figures 2 and 3. These arise among the procambial cells as well as in the cortical region. The internal layer of cells in the drawing, figure 4*C,* which is shown with nuclei, is the endodermis bounding the plerome. As this layer of cells is fol-
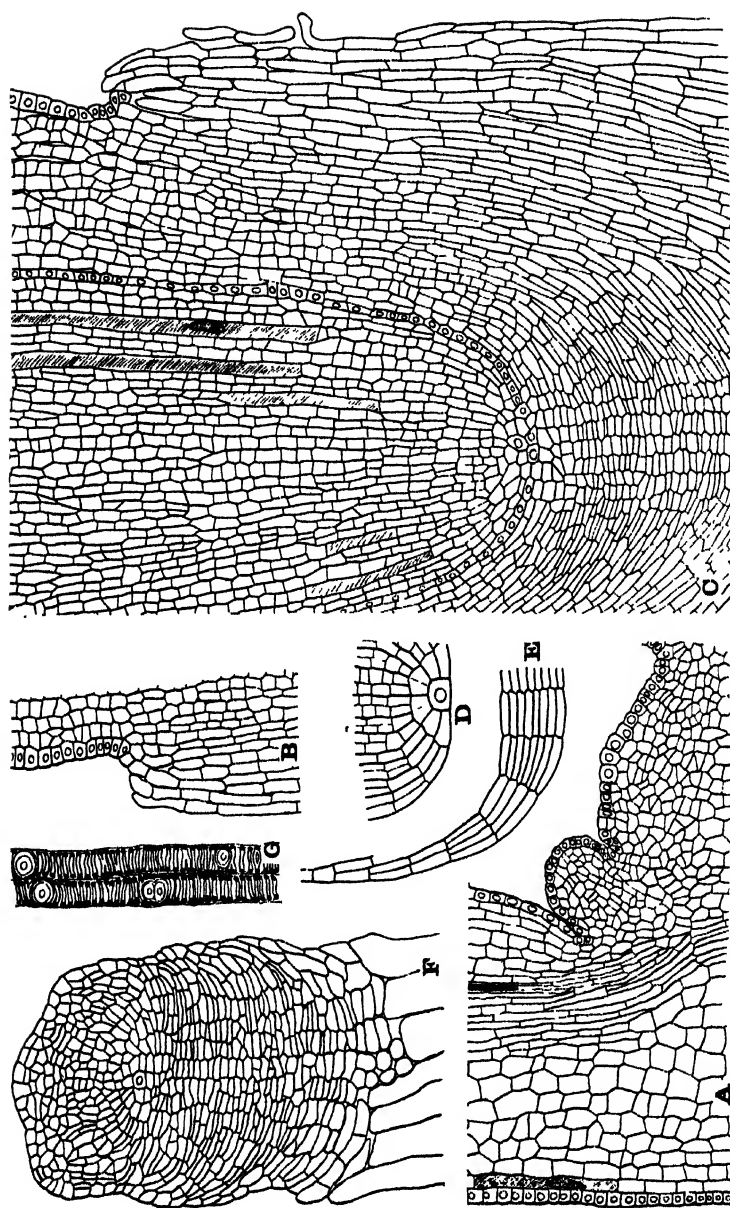
TEXT FIG. 4. A, section through stem tip primordium and base of cotyledon of the embryo of figure 2. × 100. B, section through groove on surface of embryo of figure 2. × 100. C, section through embryo of figure 2 in area C, showing lower apex of plerome, endodermis (layer shown with nuclei), and periblem above, merging with calyptroperiblem below. × 100. D, diagrammatic scheme showing arrangement of cells in plerome apex of root tip. E, scheme of cell arrangement in calyptroperiblem. F, section of early embryo showing stem tip, earliest stage of a cotyledon primordium, apex of plerome (cell with nucleus), and massive calyptroperiblem merging with elongated cells of the secondary suspensor below. × 100. G, enlarged detail of the protoxylem elements in the cotyledon of A. × 500.

lowed down to the apex of the plerome we find it ending below in a group of large cells which mark the apex of the plerome of the root.

This interesting feature may be recognized in the pine embryo and in the embryos of all conifers which we have examined. The general scheme of organization as we conceive it is shown diagrammatically in figure 4D. Usually a single cell or a small group of cells may be identified at the plerome apex from which other superimposed cells radiate upward in rows following parabolic curves. If we may think of this cell or group of cells as a single unit and let it divide in all planes in turn, it can give rise to some of the group of cells which are in the periblem below it as well as the plerome cells which overlie it. These in turn would be more restricted in their planes of division to the periclinal and anticlinal divisions necessary to create a parabloid cylinder whose lower end-section is represented in figure 4D. Of course the great regularity indicated in the scheme of figure 4D is not found in the drawing of figure 4C, for almost immediately some cells enlarge or divide more rapidly, others less rapidly, than is required in the ideal scheme, and they may crowd each other slightly out of alignment, but they remain on the whole remarkably regular in their arrangement during these early stages.

The cells in the periblem which surround the end of the plerome show a different arrangement. If we regard these rows of cells as successive skins or layers, we find that some of these layers divide and increase in number where the plerome begins to narrow. This will be seen in following downward almost any longitudinal row of periblem cells in figure 4C. Figure 4E represents in a diagram the general scheme for one of the layers of periblem where it passes down to surround the end of the plerome. A single layer of cells soon becomes a double layer and these divide to form additional layers as we pass downward, with the number of layers reaching their maximum in the column of cells immediately below the apex of the plerome. Thus the flattened disk-shaped cells outside of the plerome present sections which are elongated in a direction transverse to the long axis of the whole embryo.

At the margin of the root cap there is a conspicuous groove (fig. 1) forming a break in the contour of the outer surface of the embryo. Here the epidermis of the hypocotyl ends abruptly. Figure 4B, a drawing of an undisturbed part of the surface of the embryo, and likewise the right side of figure 4C show this transition in detail. It seems in the sections as if the single layer of the epidermis becomes split again and again into successive layers of cells, so that the epidermis or dermatogen loses its identity as a single morphological layer at this point. The epidermis therefore cannot, as in angiosperms, be followed around the periblem into the promeristem of the root tip to emerge on the opposite side as a single distinct layer. If we trace any of the rows of cells which leads inward from the epidermis its identity is completely lost among an increasing number of similar layers of cell-envelopes. It is likewise impossible to distinguish the tissues of a root cap from the tissues of the periblem. At the root tip of an embryo, we find a member which is made up

of periblem and calyptrogen combined. It is inaccurate to speak of this as a root cap; it should be regarded as a root cap made up entirely of periblem. We might therefore designate this member as the *calyptroperiblem*. Through the axis of the calyptroperiblem ending at the plerome is the column of cells which Schacht (12) and Strasburger (14) called the periblem column (periblemsäule). *Cedrus atlantica* (fig. 3) has these features in a slightly more exaggerated form than *C. libanotica.*

The cotyledons of these two species are not united into a tube at the dormant stage as reported for *Ketelecria.* This fact was ascertained by the dissection of from 20–50 seeds of each species under a 30 × binocular. The cotyledons are separate and distinct from each other for their entire length. Hill and De Fraine (7) reported cotyledonary tubes in the later stages of the embryo of *Cedrus atlantica,* which must therefore be due to zonal growth of the basal region of the cotyledons after the seed begins to germinate. It is not certain that the cotyledonary tubes observed by Hill and De Fraine were of the kind reported by Hutchinson.

Claire and Johnstone (6) have shown that in certain pines the embryo of the mature seed is small in relation to the endosperm, and in others it is relatively much larger. Their formula for this measure is the weight of the embryo divided by the weight of endosperm. From dissections of 20–30 seeds we found this value to be .407 for *Cedrus atlantica* and .413 for *C. libanotica.* For seeds of *C. libanotica* obtained from the tree at Flushing. N. Y., this value was .46. These figures are nearly three times the corresponding values for pines (6).

We have also counted the cotyledons in these embryos. In commercial seeds of *C. libanotica* the number ranged between 8 and 10; with an average of 8.8. Those from the tree at Flushing, N. Y., ranged between 9 and 14; more than ¾ had either 9 or 10 cotyledons and the average was 10.9. Cotyledons in *C. atlantica* ranged from 7–11; 8–9 was the usual number and the average was 9.

Figure 4*F* shows an embryo in an early stage with its cotyledonary primordia undeveloped or only initiated by the periclinal divisions on one of the shoulders on the side to the left of the stem tip. The elongated cells at the opposite end of the figure are embryonal tubes which form the secondary suspensor. Other cells forming the cylinder above these belong to the calyptroperiblem and those nearest the suspensor elongate in turn to add to this massive suspensor. At this stage the plerome apex of the root tip is marked by a centrally placed cell shown with a nucleus in the drawing. Fully two-thirds of the embryo is included in the calyptroperiblem, the epidermis is still undifferentiated, and the plerome is not yet sharply defined in the part which lies above the cell which marks its lower apex. There is only a shallow depression at the place where the protruding margin of the root cap will appear as the embryo develops farther.

An apical initial cell has been reported for the early embryo of *Cedrus libanotica* (4). This feature is found on the embryo of a much earlier stage and has disappeared or become too obscure to be recognized long before the stage shown in figure 4*F* has been reached.

DISCUSSION AND SUMMARY

Although the embryo of *Cedrus* does not differ from that of other Abietineae in respect to the meristematic features with which Strasburger was specially concerned, there is a well-marked break externally between the calyptroperiblem and the other portions of the embryo. All other features differ only in the relative proportions included in the cotyledons, hypocotyl, etc. According to the figures of Strasburger (14) the calyptroperiblem of *Thuja* includes fully 2/3 of the length. However, his figure (Pl. X, fig. 10) seems to include a considerable portion of the suspensor with which this structure merges, leaving about half of the length which may be considered calyptroperiblem. This is in about the same proportion as in his figure of *Pinus pumilio* (see Strasburger, Pl. XI, fig. 34). In *Pinus Banksiana* (1), this member includes about 40 percent of the length of the embryo, while only about 1/4–1/5 of the length of the embryo is included in the calyptroperiblem of *Cedrus* and *Keteleeria*. In *Cephalotaxus* (3) and *Sciadopitys* (5) this structure includes considerably less. Possibly the Abietineae all have a strongly developed calyptroperiblem and in *Cedrus* and *Keteleeria* the shortening of the calyptroperiblem has been compensated to some extent by a lateral extension of this member.

While there may be no error concerning the cotyledonary tube in the embryo of *Keteleeria Fortunei* (observed by Hutchinson from a series of transverse sections), we seriously doubt the accuracy of Hutchinson's description of the embryo with regard to " a central axis " extending throughout the length of the embryo, and shown in his figures as passing from the central part of the plerome into the axis of the calyptroperiblem; there is also some doubt as to whether the root cap may be sharply separated from the root tip at the boundary of the periblem as his diagram shows. This is certainly not possible in *Cedrus*. We regard the use of the term coleorhiza for the calyptroperiblem of a gymnosperm as inappropriate and somewhat misleading. The term coleorhiza has been used in designating the special sheath or covering of the root in the embryos of grasses, angiosperm roots which are at the same time provided with a well differentiated root cap and have very little in common with gymnosperm roots. There is, in fact, some question as to whether we should regard the calyptroperiblem as a true root cap, though this member serves this purpose.

One of the species of *Cedrus* agrees with *Keteleeria* in having primordia of the plumular leaves at this stage, but in *Keteleeria* these plumular primordia are carried up on the elongated cauline meristematic cone, while in *Cedrus* they surround a low cauline meristem which is only slightly convex. In

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Pinus this cauline meristem is decidedly convex or conical but we have found no traces of plumular primordia in the embryos of the mature seed in a casual examination of nearly a dozen species.

We have found records of only one other case in a gymnosperm aside from *Keteleeria* and *Cedrus libanotica* in which the plumular leaf primordia appear in the dormant stage of the seed. This is *Ginkgo,* in which Lyon (10) illustrated several series of plumular leaves in sections of the embryo.

Resin passages arise from cells which elongate greatly and are found in the hypodermal layer of cotyledons and hypocotyl as well as among the cells of the procambial strands.

The first vascular tissues of the embryo, spiral elements with bordered pits, appear in the cotyledons, and are in an endarch position. This is in agreement with the findings of Hill and De Fraine concerning the endarch position of the protoxylem in the cotyledons of other Abietineae. The order of appearance of the various parts as found in the differentiation of the embryo of *Cedrus* is: plerome of root tip and calyptroperiblem; stem tip; cotyledons and hypocotyl region; epidermis, covering parts of the cotyledons and hypocotyl; procambial tissue; resin passages; primordia of the plumular leaves; endarch protoxylem elements in the cotyledons.

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POLYPLANETISM AND ZOÖSPORE GERMINATION IN SAPROLEGNIACEAE AND *PYTHIUM*

WILLY HÖHNK

If we consider Ledermüller's article (1760) as the oldest report dealing with Saprolegniaceae then 109 years passed before the diplanetism of the zoöspores was discovered by Leitgeb in 1869. Leitgeb cultured a *Saprolegnia* which he believed was *Saprolegnia monoica*. But during his careful observation he found that the zoöspores escaped from the sporangium, swarmed, underwent a resting period, swarmed for a second time and then germinated or underwent a second resting period in order to germinate later when the environment offered the opportunity. This discovery lead Leitgeb to the establishment of a new genus, *Diplanes*.

The zoöspores of this new genus had different shapes during the two swarm periods. During the first they were pear-shaped and had terminal cilia, in the second one they were bean-like (kidney-shaped) and had lateral cilia. This circumstance made it easy to indicate the presence of the dimorphism in all members of the genus *Saprolegnia* (including at that time *Isoachlya*) and they believed a diplanetism to be present. Therefore after a few years the genus *Diplanes* Leitgeb had to disappear for it was shown to be synonymous with *Saprolegnia*.

The observers of the other genera of that time concluded that there was a monoplanetism in *Pythiopsis* (only the pear-shaped zoöspore with terminal cilia was reported), in *Achlya*, in *Aphanomyces*, and in *Dictyuchus* (only the bean-shaped, laterally ciliated zoöspore was found). In 1887 Hartog claimed to have shown *Achlya* to be diplanetic [1] and suggested the same for *Aphanomyces*. Nevertheless von Minden in 1915 described them again as monoplanetic, because only a very few spores in *Achlya* swarm separately either within the sporangium or in front of the hollow sphere.

The result of these studies was the formulation of the opinion which entitled us to homologize the pear-shaped, terminal ciliated zoöspore in *Pythiopsis* with that present in *Saprolegnia* and the kidney-shaped zoöspores present in *Saprolegnia, Achlya,* and *Dictyuchus*. This point of view is generally accepted and extended to the genera later established.

Diplanetism obviously played an important rôle as a taxonomical criterion among the genera and called for attention in considering the phylogeny of all the Saprolegniaceae.

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[1] Cornu in 1872 mentioned the presence of cilia when the spores left the sporangia. Similar observations were reported in several *Achlya* species by Humphrey in 1893 and Coker in 1923.

In agreement with this acceptance the genera *Dictyuchus, Brevilegnia, Thraustotheca,* and *Calyptralegnia* are supposed to have one real planetic stage, which corresponds to the second one in *Saprolegnia.* However, in 1919 Weston reported a second swarm period in *Dictyuchus* sp. The form of the zoöspore was that present in the first stage. This swarm period would be homologous to a third one in *Saprolegnia.* This, however, had not been described so far. Probably not to confuse the situation he did not speak of diplanetism in *Dictyuchus* but of repeated spore-emergence. This paper called the attention to the matter in consideration. Since then several observations have been contributed concerning the planetism of zoöspores in species of other genera. The available data are given in text figure 1.

This figure 1 is a homologization of the different swarm periods. The fourteen known genera of the Saprolegniaceae follow in their spore-behavior five types characterized by *Pythiopsis, Saprolegnia, Achlya, Dictyuchus,* and *Aplanes.* Seven examples are given in order to give a complete report of the recent observations. That part of the scheme included in the rectangle will be discussed in this paper.

However, it must be mentioned that of each of the genera concerned, only one species has shown so far a repeated spore emergence. These are *Pythiopsis intermedia* Coker and Harvey,[2] *Saprolegnia torulosa* de Bary, *Achlya racemosa* Hildebrand, *Dictyuchus* sp., and *Brevilegnia bispora* Couch. In *Thraustotheca* and *Aplanes* a similar development is not yet known. *Aplanes* is a doubtful genus, even in its new limitation as given by Coker. All species that I have studied which showed at least partly an *Aplanes*-like spore behavior also showed, due to slight changes in the environment, *Achlya*-like spore formations.

The explanation of the spore-behavior in Saprolegniaceae was generally accepted, but the situation became rather complicated when the repeated spore emergence was observed in *Pythium.* It has been reported so far in *Pythium proliferum* and its varieties by Cornu (1872), in *Pythium dictyosporum* by Raciborsky[3] (1891–92), in *Pythium diacarpum* by Butler (1907), in *Pythium Butleri* by Drechsler (1930), and *Pythium epigynum* by myself (1932). This behavior was partly explained as diplanetism, in complete agreement with the real meaning of the term.

Atkinson in 1909 described and figured a remarkable peculiarity of the spores of *Pythium intermedium,* namely, that the biciliated spores divide into two uniciliated ones. This took place during the first real planetic stage, which he explained as the second in regard to diplanetism.

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[2] Mr. Harvey completed the information by letter, saying: "In answer to your question, I shall state that the swarm-spores of the second swimming period in *Pythiopsis intermedia* apparently possess lateral cilia; the spore as I remember now and observe from notes, is kidney-shaped. It was an oversight that this information was omitted from my original paper."

[3] Obtained from citations. The original papers I have not seen.

Text Fig. 1. Homologization of swarm periods for the genera of the Saprolegniaceae.

To summarize, the term diplanetism is now applied to three cases, in which the spores are obviously of different morphological value. Applied to *Saprolegnia* it includes two different kinds of biciliate zoöspores, and applied to *Pythium* it includes either one zoöspore-form present during both swarm periods or, according to Atkinson, it may include biciliate and uniciliate spores.

The fact that diplanetism goes partly hand in hand with dimorphism has confused the situation several times as is clearly shown by Matthews (1931, p. 13) when she says: " However, since the spores in both swimming stages in *Pythium* are alike, we prefer to follow Atkinson in considering this type only as a repeated emergence rather than true diplanetism, which is found in some Saprolegniaceae."

Considering the life of the zoöspores in general as well as their homologization, it is necessary to distinguish between dimorphism and diplanetism. The latter term ($\delta i s$ = twice, $\pi\lambda\acute{a}\nu\eta s$ = swarming) means that only two movements are present regardless of the form and shape of the zoöspores. Furthermore in order to agree with the observations dealt with in this paper it must be considered that a zoöspore is able to swarm more than twice. Therefore the diplanetism is only a limited case of a polyplanetism, which is present at least in *Saprolegnia* and *Achlya,* but it is highly probable that it is a peculiarity of the zoöspores in all genera of the Saprolegniaceae.

OBSERVATIONS ON SAPROLEGNIA TORULOSA DE BARY (PLATE I)

This species was obtained from a twig taken from a spring in the vicinity of Marburg, Germany. Because of its rarity it was observed over a long period.

In regard to the homologization of the planetic stages in *Pythium* later on it is necessary to begin with the plasma differentiation within the sporangium. Also the changes within the plasma during that time should be compared with those appearing when the zoöspore leaves the membrane after the resting period.

Plasma differentiation within the sporangium

When the crosswall which separates the sporangium from the hypha was built, the next sign of the sporulation process was the formation of an outgrowth at the tip. This remained small and was developed by means of the thinning of the membrane. The plasma became differentiated into masses which were somewhat larger than the later spores and connected by delicate threads. Very soon the plasma pulled away from the membrane and the crosswall at the base curved inwards into the sporangium, showing a lessened turgor. The fine connecting threads between the spores were absorbed, and the spores now appeared smooth. The single zoöspore showed two changes which followed each other immediately. The large visible granules within each spore disappeared, and the plasma became homogenous. Instead of the granules several vacuoles occurred within each spore which, however, were

not permanent. They disappeared soon and new ones originated. Some spores showed one, but most of them two or three of these vacuoles. From the formation of the crosswall at the base until this stage which Büsgen called "homogener Zustand," and Rothert "Stadium der Trennung," one hour usually passed. However, the time depended upon the age of the culture and the condition of the surrounding water. In certain cases about twice the time was required. This homogenous condition lasted for about 12 minutes and during this time the final separation into spores took place.

Suddenly all of the small vacuoles disappeared and all of the spores aggregated at the center of the sporangium, forming there a dense body of spores. This happened, obviously, as the result of a strong pressure within the sporangium which also caused the explosive opening of the sporangium and the passive escape of the spores.

The points in consideration here are the formation of an outgrowth by thinning of the sporangium membrane and the separation of the plasma into spores which is followed by the homogenous stage. This stage results from the disappearance of the granules, which is followed by the occurrence of small vacuoles. If these latter ones disappear a pressure is acting which causes the escape.

Planetism

As soon as the mass of spores had passed the opening, it broke down into single spores. These spores offered a peculiar picture. For about 30 seconds they moved to and fro, without any sign of their own activity. They had forms like dumb-bells. Both ends were slightly swollen, in general one end somewhat more than the other. The irregular passive movement hindered the observation of a vacuole or cilia.

Then one end became distinctly thicker than the other and the spores became active. Terminal cilia were noted and near the point of their insertion there was a conspicuous vacuole. While the lower part remained quiet, the upper part made a quarter of a rotation; the whole spore thereby became twisted. The various stages were modelled in plasticine and plate 1, figures 3, 4, 5, and 6, exhibit views of the zoöspores at right angles at this stage. The heavy line, especially in figures 3 and 6, demonstrates the sharply marked fold appearing due to the turning of the tip. This fold is in reality a deepened groove and corresponds doubtless to the form of the movement present in the first swimming period. While the movement is brought about by the cilia, the fold causes a constant rotation during swimming. Also the movement of the spore in one direction is like a continuous spiral, as given in plate 1, figure 7.

The first swarming period came to an end after about 15 minutes. The movement became slower and the head of the spore (where the cilia are inserted) touched the bottom of the dish (pl. 1, fig. 8). In a few seconds the spore became spherical and a membrane was formed. Several zoöspores

behaved in the same manner when they came into contact with the surface of the water. During their resting period they remained attached to the surface. In several dishes containing cultures an enormous spore-production took place. During their resting period all the zoöspores were either attached to the bottom of the dish or to the water-surface. A tap on the table or the dish regularly caused the sinking of some spores. However, no spore was found above the surface, *i.e.*, exposed to the air; all of them stayed within the water. The presence of two membranes is therefore not necessary.

The encysted spore had no vacuole, but a few refractive granules were visible. These changed their position thereby revealing that the plasma was still in movement. This resting period in all observed cases extended over a limited time, mostly 30 minutes, often shorter, more rarely longer. A few times it had a duration of 1½ hours.

The first sign of the passing out of the plasma in order to enter the second swarm period was a little outgrowth (fig. 11), through which the plasma passed to form, in front of the orifice, a ball, first oval in shape but soon spherical. Here a vacuole appears inside which had at first an irregular outline. The plasma part outside and the vacuole inside corresponded to each other in their size. When the plasma inside pulled away from the membrane a vacuole appeared within the plasma sphere outside, which grew in proportion as the inner one became smaller. A connecting thread between both could not be seen, apparently due to its thinness. In plate 1, figure 15, the vacuole seems to be of disproportional size compared with its size within the membrane. In figure 16, it is again irregular in outline and located at the margin, where it secreted a part of its content. As a result the size of the zoöspore was reduced.

When this happened, the plasma sphere became elongated (figs. 18 and 19). The reduced vacuole stayed near the margin. Cilia were not recognized so far, but in figure 20, close to the vacuole, moving cilia became visible, gradually growing larger, and gradually their activity increased in order to loosen the zoöspore from the membrane. This zoöspore had great difficulty in breaking away from the membrane as demonstrated in figures 21–24, and 28. Strenuous efforts seemed to be made (figs. 20 and 21) but escape was impossible.

Plate 1, figures 25, 26, and 27, were taken from one of the two neighboring zoöspores. They came from the same sporangium, were also in the first resting period, but this zoöspore left the membrane. It showed two laterally inserted cilia, swarmed for 27 minutes and then underwent the second resting period.

The first zoöspore underwent the second resting period in front of the emptied spore-membrane. The cilia lost their activity gradually and disappeared. The outline became spherical again, the vacuole disappeared and a new membrane was formed. However, this resting period took only 17 minutes, during which several granules had increased in size. Then a little

outgrowth was formed and at the same time a vacuole appeared inside. The cilia became visible and efforts were made to become free, this time with success. After 16 minutes the spore swam away.

Plate 1, figures 35, 36, 37, and 38 are a generalization of the method of germination which may appear after the first as well as after the second or third swarm period. If the zoöspore germinates at once, no consistent thick membrane is formed; if it rests first, this membrane is present (pl. 1, figs. 36 and 37). One or two germ-tubes were found.

Plate 1, figures 39–45 show certain cytological conditions of uninucleate spores and of multinucleate germinated spores. They were stained *in toto* with hot carmine. Figure 39 represents a spore just entering a resting period; the elongated nucleus is connected with the cilia by its thread-like prolongation. Figure 40 corresponds to figures 15 and 30; the spore entering a swarm period has one nucleus. Figure 41 is a resting stage, showing an oval nucleus with reduced prolongation. Figure 42 might correspond to figure 11 but the two nuclei indicate that germination has taken place. Figure 43 is also a germination stage; one of the two nuclei present is in division. The length of the germ-tube does not reveal the exact number of the nuclei present (compare figs. 43 and 44). Finally, figure 45 shows a stage where the nuclei have already entered the germ-tubes.

The zoöspore described in detail above was polyplanetic. It had three swarm periods. During the first one it showed the pear-shaped, terminally ciliated form and during the last two periods the kidney-shaped, laterally ciliated form. This behavior is not due to abnormal environment, for under natural conditions the same type of behavior is to be observed.

This behavior is also present in *Achlya racemosa*. Here, under certain conditions, even a fourth swarm period was observed.

ACHLYA RACEMOSA HILDEBRAND

This fungus was collected from Lake Wingra, Madison, Wis., and the pure cultures were grown on ant-larvae. One sporangium with a part of the hypha was transferred to a plate of agar having no available carbohydrate within a petri dish. Before the agar became solid the dish was shaken; so that the agar-surface became slightly roughened. Close to the sporangium some drops of water were added and then the whole dish covered by a glass plate, its lower surface moistened with lukewarm water. Through a hole in the glass plate the objective of the microscope could be brought close enough to the object for observation. When the spores had left the hollow sphere, the hypha was taken away, for it began to branch and the formation of new sporangia was not desired.

The differentiation of the plasma within the sporangium agreed entirely with that described for *S. torulosa*. After about 70 minutes it became emptied. Normally all of the spores formed the hollow sphere in front of the orifice.

Planetism and spore germination

Concerning the escape of the zoöspores from the membrane after the first resting period there is nothing to add to the description given for *S. torulosa*. The zoöspores swam in the water and after 72 minutes I recognized the first encysting. Therewith the activity in regard to swarming should have come to an end.
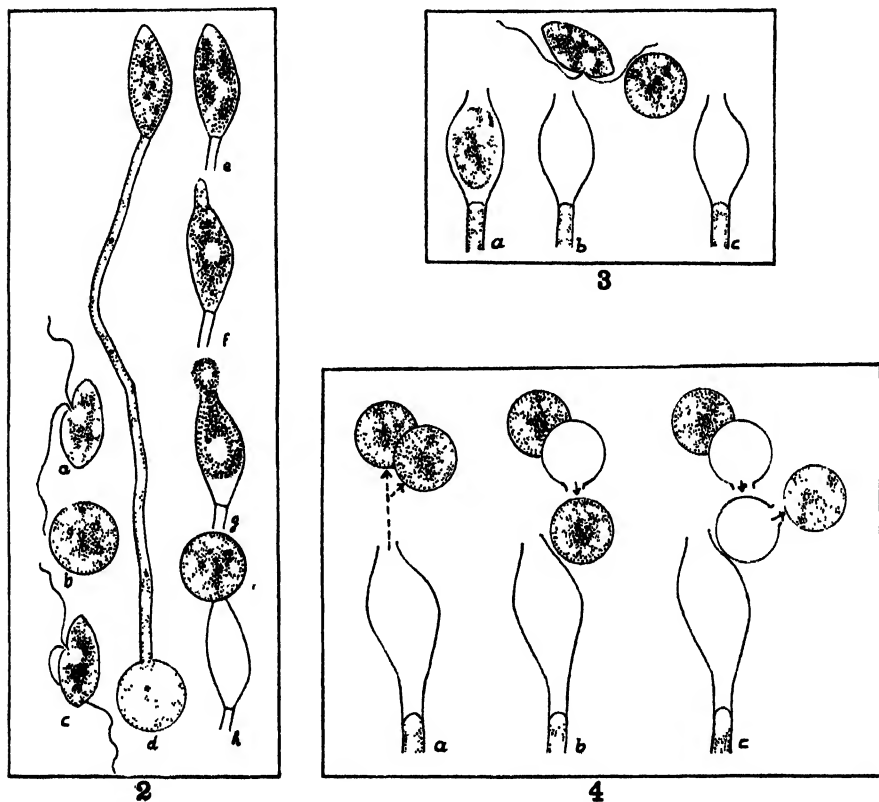
TEXT FIGS. 2–4. *Achlya racemosa*. FIG. 2. *a*, a third swarm period. *b*, third resting period. *c*, fourth swarm period. *d*, germination after the fourth resting period forming a new sporangium. *e*, *f*, development of new sporangium. *g*, escape of the plasma. *h*, new resting period. FIG. 3. Formation and escape of a swarm spore after germination. FIG. 4. *a*, germinating sporangium forming two swarm spores. *b*, encystment followed by formation of second swarm spore. *c*, encystment of second swarm spore followed by formation of third swarm spore.

After 24 minutes one of the encysted zoöspores showed a short outgrowth. However, the expected germination did not appear but the spore plasma left the membrane in the way described above. Gradually the cilia appeared and by their activity the zoöspore left the membrane without difficulty. This was actually the second swarm period of the zoöspore (text fig. 2, *a*). It would correspond to a third one in *Saprolegnia*.

The swimming zoöspore could be followed easily, for it happened fortunately that the water first added had somewhat decreased by means of evaporation or absorption by the agar. Instead of showing a continuous surface, now the water filled the little depressions of the uneven agar-plate as small droplets. This swarm period took 22 minutes; then the zoöspores encysted (text fig. 2, b).

After 1 hour and 13 minutes the plasma again left the membrane through a short tube in order to enter a third period of active swimming (text fig. 2, c) which is homologous to a fourth in *Saprolegnia*. The development of this process offered no additional feature other than that already described. The shape and size of the zoöspore as well as of the vacuole are entirely the same. About 75 minutes later its encystment took place.

This spore failed to enter any further swarm periods. After 41 minutes a small tube was formed, but its development soon showed that it would become a germ tube. This tube was about $3\frac{1}{2}\,\mu$ thick and when about $90\,\mu$ long it became club-shaped at the tip. This swelling finally had a width three times the tube diameter and the length of $18\,\mu$ (text fig. 2, d). Its development took about two hours; then a crosswall appeared at its base. The main part of the plasma was enclosed within the swelling, supposed to be a sporangium; only a small portion remained within the hypha and the membrane, including a few granules. These latter moved slowly, thereby revealing that life was present and that under satisfactory conditions it would be able to continue.

The plasma within the swelling underwent rapid translocations. At one time it appeared as if divided into four parts (text fig. 2, e) but later it became homogenous again. Text figure 2, f, drawn after about 45 minutes, indicates that the swelling was prolonged into a narrow outgrowth. Also a vacuole was present, sometimes distinctly visible, sometimes less distinct. These changes appeared in turn; the time between each was first about 70 seconds, later about 30 seconds. Text figure 2, g, about 30 minutes later, shows a distinct opening, through which the content escaped. In this case the plasma formed a sphere in front of the sporangium and became surrounded by a membrane (text fig. 2, h). The swelling proved to be a sporangium containing one zoöspore.

Together with this spore, within the same water droplet were four other spores. One of them is drawn in text figure 3. How often it swarmed was not determined. It germinated earlier than the spore described above. At the tip of the germ tube a similar swelling was formed, containing also only one zoöspore, which stayed for almost $\frac{3}{4}$ of an hour within the sporangium. Then a vacuole became visible and close to it a marked constriction, probably the place of insertion of the cilia (text fig. 3, a), but these became distinct only when the zoöspore had escaped. It was kidney-shaped and had two laterally inserted cilia (text fig. 3, b). It swarmed slowly, influenced probably by the decrease in the water present. Its movement could be followed

easily.   The spore described a few circles and then it stopped near the mouth of the sporangium, became spherical, the cilia disappeared, and soon a surrounding membrane was built (text fig. 3, c).

The surprising thing is, that a fully developed zoöspore left the sporangium and had the kidney-shaped form instead of the pear-shaped, and also that it left without entering a resting period in front of the sporangium opening.   Probably the long stay within the sporangium when the latter was already opened corresponded to the first resting period.

Because the single spores were scattered on the agar, in the other small water droplets many similar stages could be found.   Some of them are given in text figures 4, 5, and 6.

Text figure 4 is shown because the sporangium formed two zoöspores. Both escaped immediately.   They had the elongated form and clung together while active cilia could not be indicated.   After 1½ minutes they became spherical and encysted (text fig. 4, a).   One of the spores escaped again after about 20 minutes (text fig. 4, b).   The mode of its escape and whether it formed cilia were not observed.   However, 40 minutes later the same spore escaped again (text fig. 4, c) in the same way as described for *Saprolegnia torulosa;* but cilia could not be determined.   This indicates that the escaping spores do not necessarily swarm.

Text figure 5 shows two new facts.   The plasma during escape developed within the sporangium a large vacuole, moved through the opening, became
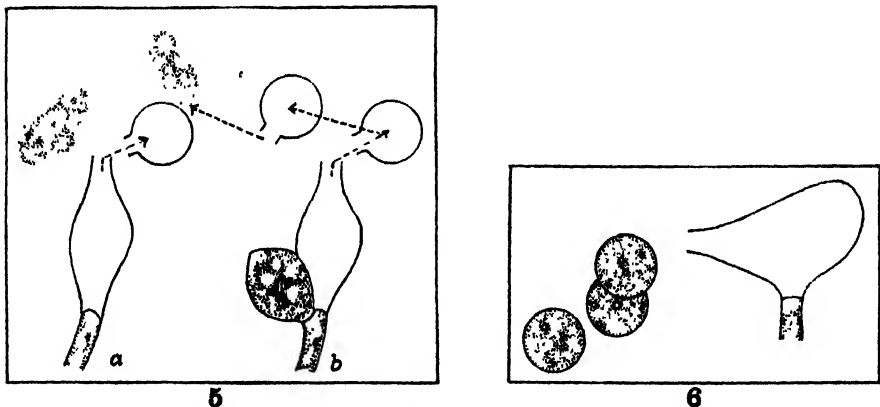
TEXT FIGS. 5 and 6. *Achlya racemosa.* FIG. 5. a, sporangium forming amoeboid swarm spore. b, germination after encystment of the amoeboid swarm spore shown in a. This figure also shows the formation of a second sporangium beneath the first. FIG. 6. Sporangium liberating three zoöspores.

spherical, and underwent a resting period; but the emerging plasma neither swarmed nor became spherical.   Its outline was elongated and its movements were that of an amoeba.   Text figure 5, a, shows this stage.   Here, at the lower surface, even a pseudopodium is visible.   It moved very slowly and only for a short distance, as can be seen in text figure 5, b.   After about 25

minutes it became enclosed in a membrane, but later it left the membrane and the amoeba-like form and movement appeared for the second time. The structure of the plasma was similar to that of the zoöspore. It included a few refractive granules of small size and a vacuole. The location of the latter, however, was different (see text fig. 5, *a* and *b*). Also its size changed, not only in different stages but also during its amoeboid stage. The second new fact is the presence of a second sporangium from the same germ tube. It was developed laterally, in the manner typical for *Achlya*. In all the observed sporangia a proliferation was never recognized.

Doubtless the lack of water, which had dried up in the meantime, caused the amoeboid movement. Nevertheless the zoöspore had the ability to germinate, to swarm, and to creep in amoeba-like fashion, and reacted to environmental conditions in any of these stages.

It would be of interest to learn whether these amoeboid stages are uni- or multinucleate. The plasma bodies observed here did not increase in size, at least not in an amount which could be measured, for the diameter of the second cyst in figure 5, *b*, was not longer than that of the other membranes, which would suggest that in these cases only one nucleus was present.

Text figure 6 was an exception to the usual behavior. The sporangium was irregular in shape and contained three zoöspores.

To avoid the death of the culture some drops of water were added but these caused a disturbance in the location of the spores. I failed to find the observed ones definitely. However, within the next five minutes many swimming zoöspores crossed the field and within 15 minutes most of the encysted spores had entered a swarm period.

As the result of the observations in *Achlya racemosa* it may be said that here polyplanetism is better demonstrated than in *Saprolegnia torulosa*. Spore germination is also well distinguished from sporulation. Sometimes it appeared as if the membrane of the germ tube was not a prolongation of the cyst membrane. But the demonstration by means of plasmolysis failed.

<div align="center">PYTHIUM</div>

Both species mentioned below were obtained from soil samples from the vicinity of Madison, Wis.

Atkinson (1909, p. 450) said the " formation of the spore-origins in the prosporangium and their migration to the outside, I believe, represents the first swarming period, and if so the zoöspores of *Pythium* are diplanetic and *Pythium* then represents a simpler and more generalized condition of this phenomenon than is found in *Saprolegnia*." Atkinson concludes that we have to compare the development of the spores within the sporangium in Saprolegniaceae and *Pythium*. That in *Saprolegnia* has already been given, and the characteristic stages in *Pythium* will next be mentioned.

Plasma differentiation within the sporangium in P. proliferum

The sporangia were mostly terminal, seldom intercalary.   When the swelling reached final size, they became separated by crosswalls.   The plasma was heterogenous, and contained many relatively large granules.   Regardless of whether sporulation took place at once or after several weeks an exit tube was formed immediately about as long as the diameter of the sporangium. Its formation was associated with the appearance of a distinct large vacuole, which had been present throughout the whole dormant period.

The sporulation depended upon environmental conditions.   The time required for the process sometimes was about 20 minutes, sometimes more than two hours.   However, in each case the following story was repeated: The plasma became differentiated into parts somewhat larger than the final spores. Their outlines were angular, but the separation was not complete, for connecting threads were visible.   The separating membranes were of varying visibility in different sporangia.   The next stage was the pulling away of the plasma from the membrane and during the following minutes the granules, especially the larger ones, disappeared.   Each plasma part (" spore origin ") now contained one or a few small vacuoles, which, however, were not permanent.   A contraction of the whole plasma followed; the vacuoles disappeared and the discharge took place.   The tip of the exit tube was distended into a thin vesicle, growing in correspondence to the amount of plasma flowing into it.   The fact that in several cases the discharged plasma within the vesicle had an irregular outline suggested that the former differentiation into spores was still present.   However, more frequently the discharged plasma did not seem to be subdivided.   The homogenous stage, starting within the sporangium, where the granules were dissolved, was found here to be within the vesicle.   Then the final formation of the zoöspores took place, the vesicle broke, and the zoöspores swarmed away.

In all these characteristic phases, the spore development in *Pythium* agrees with and corresponds to that in *Saprolegnia*.   Therefore there is no reason for interpreting the discharge of the " spore origins " as a first stage of a diplanetism.   Or if we do so in spite of the result of such a comparison, then we must explain the plasma differentiation into " spore origins " in *Saprolegnia* and *Achlya* as a first stage and therefore in *Saprolegnia* at least a triplanetism would be present.

Planetism in P. epigynum

However, in spite of that interpretation repeated spore emergence in *Pythium* occurs, which must be considered as a diplanetism or as a type case of a probable polyplanetism.

In two cases observed, a zoöspore formed an exit tube by means of thinning of the extension of the consistent membrane.   The one was $4\,\mu$, the other one $12\,\mu$ in length.   The plasma followed the growing tip at a short distance.   In correspondence to the growth a vacuole appeared within the

spore plasma which subdivided into two when the plasma, breaking the thinned membrane at the tip of the tube, slowly escaped, forming a sphere at the outside. Parallel with this process the refractive granules decreased in number and probably also in size. The flowing out took 19 and 16 minutes, respectively, the pause at the orifice 17 and 32 minutes, respectively. During this time the vacuole was reduced, the spore became elongated, and the movement of the cilia caused the departure as a biciliate zoöspore. The movement could not be followed, and therefore it cannot be decided whether these spores entered a third planetic stage.

DISCUSSION

Spores react to environmental conditions by means of germination, swarming, encystment, or amoeboid movement. The main factors for the appearance of any one of them are the amounts of available food and water. Germination depends more upon food than water. It always takes place when sufficient food is present and at least a minimum amount of water. Resting and swarming appear within a relatively large amount of water which dilutes the food too much to attract the zoöspores and stimulate growth. Amoeboid movement, however, seems to be related to unsatisfactory water conditions. From these points of view the observations described above can be explained. In addition to food and water, other stimulants seem to have some influence as suggested by Couch (1926). However, the limits between the two or three environmental components still have to be determined.

The germination of spores may appear during each resting period, as illustrated in text figure 1. Thus *Saprolegnia* spores can be forced to germinate during the first encystment, which is described as the habit in *Pythiopsis*. Experiments have shown that a resting condition is a prerequisite to either germination or swarming. Spores of several Saprolegniaceae were transferred to nutrient agar; they escaped from the sporangium and swarmed for an exceedingly short time but then rested, sometimes without forming a membrane, for 10–20 minutes, and then germinated. The developing spore germ tube or germ mycelium is of small diameter, which remains almost the same in spite of changes in the food concentrations within the agar. Hyphae or gemmae germ tubes, for example, react in their diameter to the food concentration. For certain species a nutrient agar can be built up within which the fungus develops in the same shape and size as within water, also forming vegetative and sexual organs. The delicate appearance of the germ tube and the fact that in certain earlier observations sporangia were formed at their tips which contained only one spore, probably caused this process to be interpreted as repeated emergence or as "indirect" diplanetism. However, in addition to this occurrence (text fig. 2) in *Achlya racemosa* sporangia were formed which contained two (text fig. 4) or even three (text fig. 6) spores and in text figure 5 even a second sporangium was formed at the same germ tube. This means that growth and multiplication really took place, and

therefore it is correct to call this process germination or better, in considera-
tion of the delicacy of the tubes, to express it as spore germination. The
amoeboid movement is obviously homologous to a swarm period, for at the
start as well as at the end there is an encystment. The importance of a
resting condition has already been mentioned. It has probably two functions.
On the one hand it preserves life over a period of unfavorable conditions and
on the other hand, especially when no consistent membrane has been formed,
it probably represents a preparation stage, after which germination may take
place.

Since *Pythium* does not represent a simple case of diplanetism (dimor-
phism), as suggested by some authors in order to derive the Saprolegniaceae
from unicellular organisms, it is worth while to see whether polyplanetism or
dimorphism present here requires such origin.

Polyplanetism is not entirely a matter of the behavior of a single spore.
The species as individuals have the ability to react to different external con-
ditions. As long as the members of this fungus group were collected from
water, only species with distinct planetic stages were known (except *Aplanes*).
Since members of the group have more recently been obtained from soil, we
know that many of these forms develop sporangia and spores, but the latter
in general do not swarm. As we go from the water forms towards the land
forms there is a pronounced tendency from planetism to aplanetism, as
shown in text figure 1. This change with regard to spore behavior is demon-
strated in the five types figured. The 14 known genera of the Saprolegni-
aceae belong to one or the other of these groups. In order to give a further
illustration that these types really represent adaptations to the environment
the 14 genera are here grouped accordingly. The numerals in parentheses
indicate the number of species of each genus, the other the number of species
found in soil.

Type I.
    Pythiopsis ....(3)　1

Type II.
    Saprolegnia ....(24)　1
    Isoachlya ....(4)　1
    Leptolegnia ....(3)　1

Type III.
    Achlya ....(27)　10
    Aphanomyces ....(9)　3
    Plectospira ....(1)　—
    Protoachlya ....(1)　—

Type IV.
    Dictyuchus ....(5)　3
    Brevilegnia ....(5)　5
    Thraustotheca ....(2)　1
    Calyptralegnia ....(1)　1

Type V.
    Geolegnia ....(2)　2
    ?Aplanes ....(4)　—

The evidence becomes still plainer when we consider how often the species
concerned were found in soil, especially those belonging to the intermediate
types. For example, *Saprolegnia ferax* was found once, *Isoachlya moni-*

lifera once, but *Achlya flagellata* 16 times [4] and 68 times [5] and *Achlya caroliniana* 27 times.[4]

Further evidence becomes available when we study the known ecological data as to the kind of soil, its water content, the depth, etc.

These results might be only a parallelism, but the fact that certain of these soil fungi have shown the swarming zoöspores, which in general are lacking, demonstrates that the ability to react differently is only dormant and that the general habit is the result of more or less constant external conditions. According to this point of view the five types of spore escape from the sporangia are reactions or adaptations to different environments. The types I and II occur chiefly in water, type III under swampy conditions, and the types IV and V represent progressive steps towards typical soil conditions.

If this interpretation holds, then it is possible to explain the development of dimorphism in a similar way, as originating and progressing within the limits of the Saprolegniaceae. Thus there exists no necessity to derive polyplanetism or dimorphism from other groups of fungi, and " diplanetism " is not evidence for the evolution of the fungi concerned from Chytridiales.

The pear-shaped terminal ciliated zoöspore form is identified with the types I, II, and III. The line of progressing development probably goes from *Achlya* through *Isoachlya,* and *Saprolegnia* to *Pythiopsis.* In this progress we find other morphological characters appearing and can apply a phylogenetic principle used among the higher plants, *i.e.,* that oögonia containing many oöspores are more primitive than those which contain only one oöspore. Accepting this viewpoint, *Achlya* represents a simpler form than *Pythiopsis;* therewith there is a reason for explaining the dimorphism by means of an extension of the stage showing the pear-shaped terminal ciliated zoöspore. Parallel with this development in the three types mentioned, an ecological change takes place from swampy conditions to water associations.

Finally there arises the question of the phylogenetic value of the two forms of zoöspores. The facts that the kidney-shaped spores with lateral cilia appear in almost all the genera (except *Geolegnia* and *Aplanes*) and that during repeated emergence they are again present, indicates their importance and significance. The pear-shaped spores, however, can be interpreted either as an adaptation phenomenon or as a new acquisition. The latter spore form is the earliest, the other the final form in the life of the zoöspore. While the earlier form has some taxonomic value within the family, the final form has phylogenetic value for this group of fungi. This conception agrees with the suggestion of Marshall Ward (1883), Humphrey (1893), Butler (1907), Atkinson (1909), Scherffel (1925), and others. Also the suggestion (Marshall Ward, Humphrey) that the earlier form has the task of emptying the sporangium and the final form takes care of the distribution is acceptable throughout in the case of *Achlya.* However, in the same measure as the ex-

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[4] Taken from Harvey, 1928.

[5] Taken from Raper, 1928.

5

tension of the preliminary swarm period takes place, this form has to solve both tasks, *i.e.,* emptying of the sporangium and distribution of the spores.

SUMMARY

It is necessary to distinguish between planetism and dimorphism of the zoöspore. The application of the expression "true diplanetism" is confusing.

Planetism is a polyplanetism of which the diplanetism is a certain phase.

The comparison of plasma differentiation within the sporangia in *Pythium* and the Saprolegniaceae does not support the interpretation that in *Pythium* it represents a homolog to the preliminary swarm stage in *Saprolegnia*.

Planetism as well as the types of spore discharge can be explained by means of adaptation to environmental conditions. Under certain circumstances the zoöspores may undergo amoeboid movements, which is not a characteristic of uniciliate spores (Gäumann, 1926).

Spore germination is well distinguished from planetism. The term "indirect diplanetism" does not agree exactly with the observations made.

Dimorphism is a taxonomic criterium within this family, but the phylogenetic view should put more emphasis upon the final zoöspore form.

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EXPLANATION OF PLATE 1

SAPROLEGNIA TORULOSA de Bary

Fig. 1. Escaping zoospore, dumb-bell-like.

Fig. 2. After about 30 seconds, pear-shaped.

Figs. 3 6. Views of the motile spore during the first swarm-period.

Fig. 7. Corkscrew-like movement.

Fig. 8. The zoospore, cilia attached to the bottom of the dish, entering encystment.

Fig. 9. Encystment, a few seconds later.

Fig. 10. 12:25 P.M., the same.

Fig. 11. 12:34 P.M., escaping zoospore entering the second swarm-period.

Figs. 12–14. 12:36 P.M. 13. 12:36'30″ P.M. 14. 12:37 P.M.

Fig. 15. 12:37'30″ P.M.

Fig. 16. 12:38 P.M. Note vacuole near the margin.

Fig. 17. 12:40 P.M., reduced vacuole.

Figs. 18, 19. 12:41'30″ P.M. Change of shape. 19. 12:42 P.M.

Fig. 20. 12:44 P.M., becoming active.

Fig. 21. 12:46 P.M. Gliding of the spore.

Fig. 22. 12:53 P.M.

Figs. 23, 24. 1:08 P.M. 24. 1:10 P.M., departing zoospore.

Fig. 25. Free but remaining in place.

Fig. 26. Cilia movement and departure.

Fig. 27. 1:13 P.M., second resting period.

Fig. 28. 12:55 P.M. Corresponds to fig. 22.

Fig. 29. 1:00 P.M. Second encystment, 17 minutes.

Fig. 30. 1:19 P.M. Third swarm period.

Figs. 31-33.   1:20 P.M.   32. 1:28 P.M.   33. 1:33 P.M.

Figs. 34, 35.   1:35 P.M.   35. Becoming spherical.

Figs. 36, 37.   Germination after membrane formation.

Fig. 38.   Germination without membrane formation.

Fig. 39.   Spore with an elongated nucleus.

Fig. 40.   Escaping zoöspore.

Fig. 41.   Resting period.

Figs. 42-45.   Multinucleate stages of germination.

W Höhnk, del.

HÖHNK: SPORE FORMATION
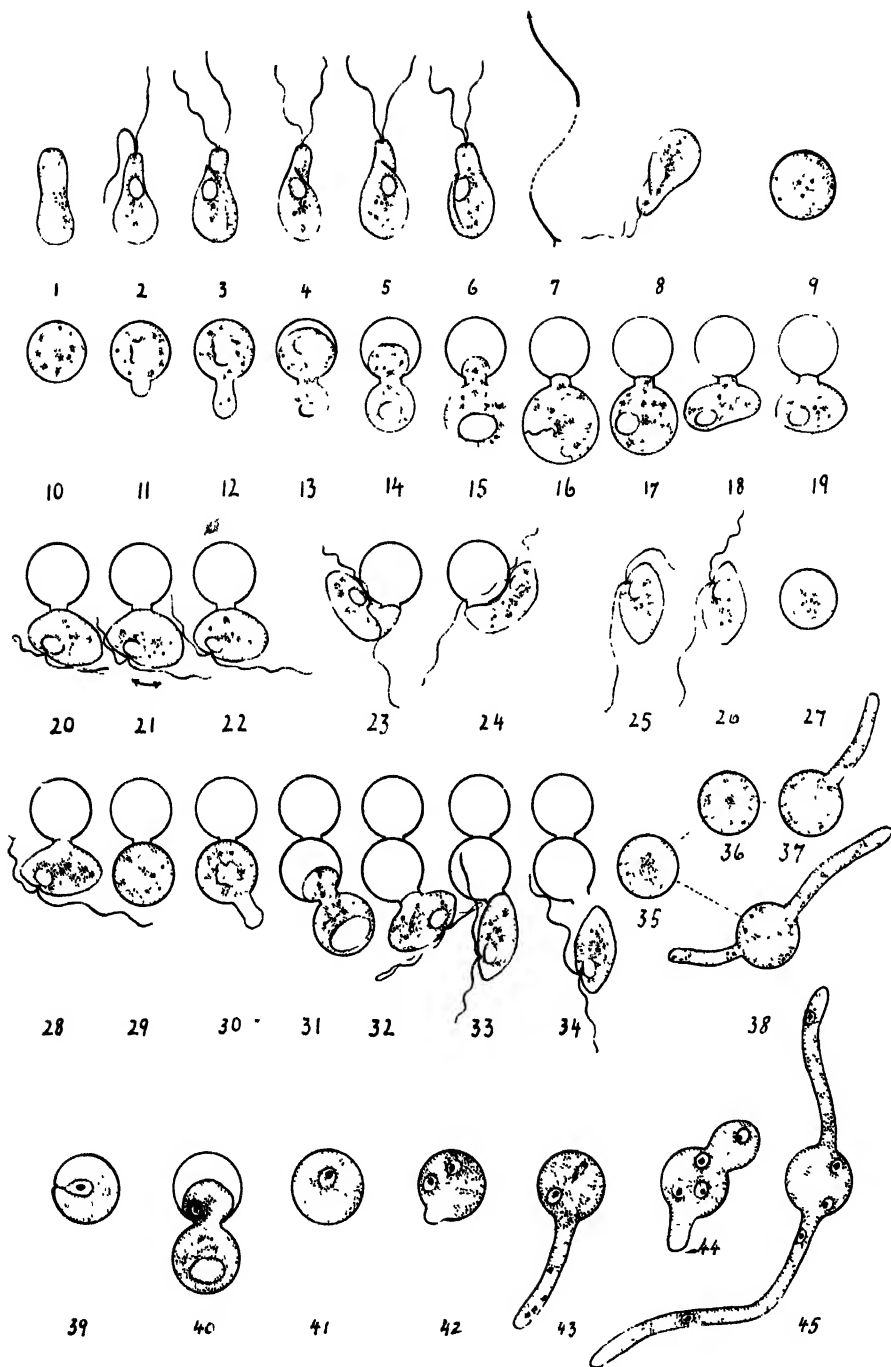
OBSERVATIONS ON OPERCULATE CHYTRIDIACEOUS FUNGI COLLECTED IN THE VICINITY OF ITHACA, N. Y.

F. K. Sparrow, Jr.

During the year 1931–32 the writer collected a number of aquatic fungi in the vicinity of Ithaca, N. Y., with the idea of adding to the knowledge of the little-known phycomycetous flora of that region. Of these, in the present paper are described those members of the Chytridiales which liberate their zoöspores from the sporangium after the dehiscence of an operculum. The inoperculate forms, as well as members of other aquatic groups, will be described in a future paper.

In grouping these organisms on the common characteristic, the possession of an operculum, the writer does not mean to imply—at least for the present—that they are interrelated. They were so placed chiefly as a matter of convenience. Nevertheless, in working with these forms, the operculum impresses one as a morphological structure of some significance. In view of the absence in most forms of unquestioned data on sexuality, the writer is at present uncertain to what extent such a grouping is justified. It must be acknowledged, however, that the recognition of the method of sporangial discharge in the chytrids as being taxonomically important, can hardly result in more chaos than has attended their classification according to thallus structure.

MATERIALS AND METHODS

The fungi herein described were all collected in the vicinity of Ithaca, N. Y. Culture and collecting methods similar to those described in a previous paper (10) were employed.

In attempting to identify these species it becomes increasingly apparent that descriptions in taxonomic works or original papers, even though they be very complete, are nearly valueless when specimens are not illustrated. Numerous figures are therefore included so that in the future there can be little question as to just what type of organism was observed.

SPECIES COLLECTED

1. *Chytridium versatile* Scherffel, Arch. Protistenk. **54**: 177, Pl. 9, figs. 17–20, 1926.

Sporangium extramatrical, smooth-walled, pyriform, slightly expanded in the proximal region to which there is attached a single, tenuous rhizoid which penetrates the valve of the diatom; in smaller plants, 12μ tapering to 3μ in diameter by 20μ in length (text fig. 1, *a*); in large plants measuring 25μ in

diameter $\times$ 30–35 μ in length (text fig. 1, m) ; opening by a single apical operculum, 8–10 μ in diameter. Zoöspores probably fully formed within the sporangium, liberated in an evanescent vesicle or " slime " (text fig. 1, b, c) ; uniciliate, 3–5 μ in diameter with a single oil globule about one-half the diameter of the spore in size. Resting spores not observed.

Parasitic in *Navicula* sp. (?), Mud Pond, McLean, N. Y., Oct. 1931.

Sporangia of two sizes were observed in this material. In the larger, broadly pyriform sporangia, the intramatrical rhizoidal system could be distinguished within the host (text fig. 1, m) whereas in the smaller plants the tenuity of these threads probably rendered them invisible.

Scherffel did not observe the emergence of the zoöspores in his material and hence could not determine the genus of the fungus with certainty.

2. *Chytridium sphaerocarpum* Dangeard, Amend. Le Bot. **2**: 244, Pl. 17, fig. 9.

Sporangium extramatrical, smooth-walled, broadly to narrowly pyriform (text fig. 1, g, h), asymmetrical; 10–18 μ long by 7–8 μ in diameter; with an extremely tenuous, scarcely if at all branched rhizoidal system of varying length and a prominent papilla surmounted by an operculum 3–5 μ in diameter. Zoöspores fully formed within the sporangium; spherical, uniciliate, 3–5 μ in diameter, with a single oil globule; escaping fully formed after the dehiscence of the operculum (text fig. 1, i). Resting spores not observed.

Parasitic on *Spirogyra* spp. (?) and *Stigeoclonium* sp. (?), Bessemer, N. Y., Jan. 1932.

When first observed this fungus was considered to be a species of *Rhizophidium*. It was only after zoöspore discharge was observed under oil immersion and very favorable conditions of light that the presence of an operculum was detected.

In the shape of its sporangium the present fungus resembles to a degree the organism termed *Chytridium mamillatum* by Braun (2), although not so citriform as the latter. However, while not describing the actual exit of the zoöspores, Braun does state that the prominent apical " Warze " is not marked off as a lid, as in *C. olla*. In the figures of *C. mamillatum* found by Pringsheim on *Stigeoclonium* and described in Braun's paper, the former investigator definitely states that he did not observe the discharge of the spores. This fungus was later found by Schenk (9) on *Ulothrix*, but here again, discharge of the sporangium was not observed. The species has been transferred to *Rhizophidium* by Fischer (6). However, in view of the extreme tenuity of the walls of the operculum exhibited by the present material, the writer is not fully convinced of the justification for this action.

In 1891 Dangeard (5) described as *C. asymmetricum* a form on *Tribonema* very similar to the present fungus, which has been referred to *R. mamillatum* (Braun) Fischer by Fischer. However, zoöspore discharge is very doubtfully alluded to and it is not certain just how it did take place. In *C.*

sphaerocarpum Dangeard, the closest resemblance is found to the American material. The pyriform, slightly tilted or asymmetrical sporangium possesses a single unbranched rhizoid and a very prominent papilla. Further, the zoöspores are liberated after the dehiscence of an operculum. Dangeard considered his fungus similar to *Rhizophidium sphaerocarpum* (Zopf) Fischer (at that time *Rhizidium sphaerocarpum* Zopf). While Fischer mentions Dangeard's plant in his discussion of Zopf's fungus and points out that it is a *Chytridium*, no further mention seems to be made of it under the latter genus.

Certain of the American plants, particularly those shown in text figure 1, *h*, show a marked resemblance to *Rhizophidium minutum* Atkinson. Atkinson (1) states, however, that his fungus discharged its zoöspores in an inoperculate fashion. *R. Sciadii* Zopf (14, figs. 27–29, 31*g*), *R. granulosporum* Scherffel (*l. c.*, fig. 81*a*), and *R. sp.* (?) on *Zygnema* Scherffel, also have sporangia similar to those of the present fungus.

Since Dangeard's *Chytridium sphaerocarpum* is the only fungus with similar sporangia which is described as discharging its zoöspores after the dehiscence of an operculum, this binomial is applied to the present material.

3. Chytridium inflatum n. sp.

Rhizidium lagenaria (Schenk) Dang., Le Bot. 1: 64, Pl. 3, fig. 23, 1889.

Sporangium extramatrical; smooth-walled; broadly pyriform or urn-shaped, with a prominent papilla (text fig. 1, *k*) surmounted by an operculum; connected by a narrow cylindrical isthmus to a spherical, intramatrical, sub-sporangial portion which is devoid of rhizoids. Zoöspores fully formed within the sporangium; uniciliate, having a single oil globule. Resting spores not observed. Plants falling into two size classes were observed, both occurring on *Cladophora:*

1. Sporangium 15 μ in diameter by 17 μ in length
   Intramatrical swelling 9 μ in diameter
   Operculum 6 μ in diameter
   Zoöspores 5 μ in diameter, with a relatively small oil globule
2. Sporangium 7 μ in diameter by 10 μ in length
   Intramatrical swelling 5 μ in diameter
   Operculum 4 μ in diameter
   Zoöspores 3 μ in diameter, with a relatively large oil globule

Parasitic on *Cladophora* sp. (?), Fall Creek, Ithaca, Sept. 1931.

Sporangiis piriformibus et operculatis, 7–15 μ diam. × 10–17 μ longis, vesicula infrasporangiali intramatricali praeditis, rhizoidibus non observatis; zoösporis globosis, 1-ciliatis, 3–5 μ diam., uniguttulatis; sporis perdurantibus ignotis.

Hab. in *Cladophora* sp. (?), New York.

Upon the dehiscence of the operculum (text fig. 1, *j*, *l*) the zoöspores emerged *en masse*, seemingly stuck together by their cilia. After a few mo-

ments at the mouth of the sporangium, they became separated from each other by an intermittent jerking and darted away.

This fungus was first assigned to *C. lagenaria* Schenk. However, as
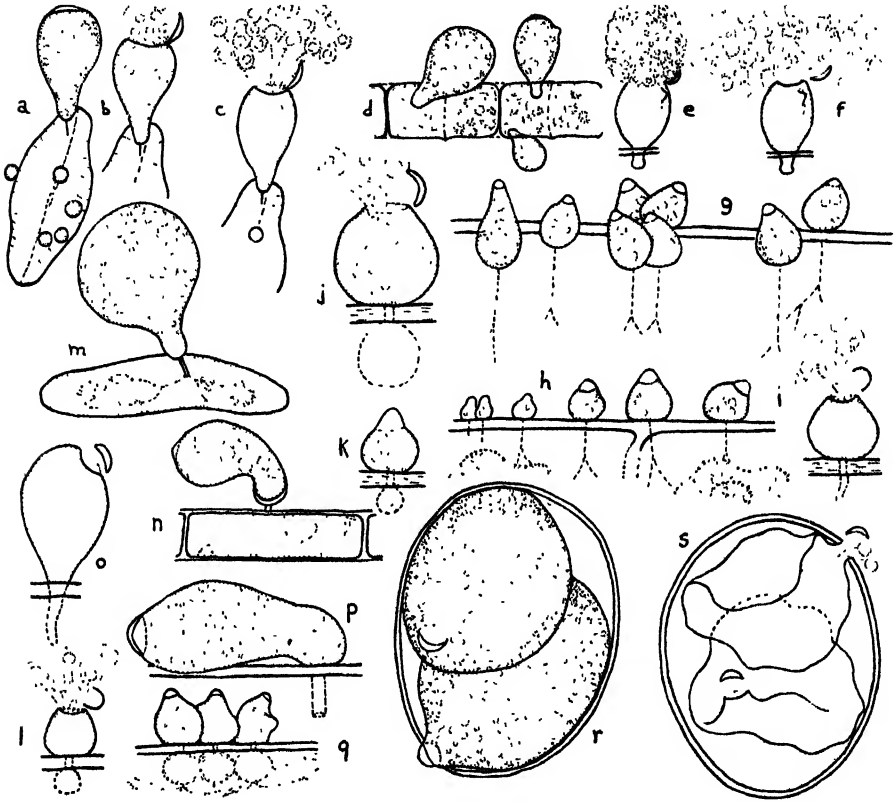
TEXT FIG. 1, *a–s. Chytridium versatile. a,* Mature sporangium and cystospores on *Navicula* sp., × 600; *b,* Dehiscence of operculum and early stage in emergence of zoöspores, × 600; *c,* Liberation of zoöspores, × 600; *m,* Large sporangium on a species of *Navicula,* × 600.

C. epithemiae. d, Habit of fungus on filament of *Melosira,* × 600; *e,* Emergence of zoöspores, × 600; *f,* Dispersal of zoöspores, × 600.

C. sphaerocarpum. g, Narrowly pyriform sporangia on *Spirogyra,* × 600; *h,* Younger and more broadly pyriform sporangia on another species of *Spirogyra,* × 600; *i,* Discharge of zoöspores, × 900.

C. inflatum n. sp. *j,* Large sporangium on *Cladophora* discharging zoöspores, × 900; *k,* Habit of small sporangium on same host, × 900; *l,* Same sporangium discharging zoöspores, × 900.

C. curvatum n. sp. *n,* Habit of plant epiphytic on *Stigeoclonium* (?), × 900.

Chytridium sp. (?). *o,* Empty sporangium on *Cladophora,* × 600.

C. appressum n. sp. *p,* Habit of plant on *Melosira,* × 2000.

C. Schenkii. q, Habit of sporangia on *Oedogonium,* × 600.

Endochytrium (?) *oöphilum* n. sp. *r,* Undischarged, mature sporangia in Rotifer egg, × 600; *s,* Same after discharge of zoöspores; two zoöspores just emerging, × 600.

will be pointed out later, this species appears, to the writer at least, to be untenable.

As originally described by Schenk[1] (9), *Chytridium lagenaria* was an inoperculate form having an ovate sporangium with a blunt, rounded apex and a larger, broadly elliptical, somewhat flattened intramatrical, subsporangial portion from which rhizoids emanated. In 1889, Dangeard (4) described under the binomial *Rhizidium lagenaria* (Schenk) Dang. a somewhat similar appearing fungus in which the extramatrical part of the sporangium equalled or exceeded the inner portion, from which stout, branched rhizoids arose. However, this fungus was described and figured as possessing an operculum. It was placed by Dangeard in *Rhizidium*, in the older sense of that genus, by reason of the presence of an intramatrical swelling; no comment was made on the operculate character. On the strength of Dangeard's observations, Schenk's fungus was replaced in *Chytridium* by Fischer (*l. c.*) and later by von Minden (8). The fungus was also observed by Wildemann (12).

Schenk correctly considered his organism closely allied to *Phlyctochytrium* (*Chytridium*) *Hydrodictyi* (Braun) Schröter. It differed from the latter chiefly by the possession of rhizoids on the sub-sporangial swelling. Fischer, however, seems to have erred in placing Schenk's fungus back in *Chytridium* because of Dangeard's observations. The two latter investigators were working with different fungi; one with an inoperculate form similar to *Phlyctochytrium hydrodictyi*, the other with an operculate form similar to the American fungus. In view of the variations in other chytrids observed by the writer in the shape of the sub-sporangial base and the extent of the rhizoidal system, he is inclined to refer Schenk's fungus to *P. hydrodictyi*. Since the present rules prohibit the use of the name *Chytridium lagenaria*, Dangeard's fungus and the present one are segregated under a new binomial.

4. *Chytridium Schenkii* (Dang.) Scherffel, *l. c.*, p. 237, Pl. 10, figs. 125–129; Pl. 11, figs. 130–132.

     Rhizidium Schenkii Dangeard, Ann. Sci. Nat. VII, **4**: 297, Pl. 13, figs. 24–30.

     Phlyctochytrium Schenkii (Dang.) Schröter, Engler u. Prantl, Natur. Pflanz. 1^1: 78.

     Rhizidium intestinum Schenk pro parte, " Vorkommen contractiler Zellen etc." Wurzburg, 1858.

A description of this species has recently been given by the writer (10). In the present material the sporangia were more irregular in shape (text fig. 1, *q*) than those previously encountered. Save for the constant presence of

[1] His rare " Contractiler Zellen etc." paper was recently seen at the British Museum. He there describes another, operculate, fungus as *C. lagenaria*, on *Nitella*. While thus, later, amending his original account, it is certain that the two fungi are different. Discussion of the second form, Dangeard's species, and " Rhiz." Westii Massey, will be published later.

rhizoids, the less prominent papilla, and the method of zoöspore discharge (in a vesicular structure), the Ithaca material might well be referred to the previously described species (*C. inflatum*). No resting spores were observed.

Parasitic on *Oedogonium* sp. (?), Fall Creek, Ithaca, N. Y., Sept. 1931.

5. *Chytridium epithemiae* Nowakowski, Cohn, Beitr. Biol. Pflanz. **2:** 82, Pl. 4, figs. 12, 13.

Sporangium extramatrical, with a lateral protuberance (operculum ?) (text fig. 1, *d*); ovate to pyriform; the basal, cylindrical, intramatrical portion slightly expanded at its terminus which is devoid of rhizoids; 6–13 μ in diameter by 10–15 μ in length; provided with an apical operculum about 5 μ in diameter (text fig. 1, *d*). Zoöspores probably fully formed within the sporangium, emerging upon the dehiscence of the terminal operculum and forming a compact, spherical mass (text fig. 1, *e*) at the mouth of the sporangium before dispersing (text fig. 1, *f*); uniciliate, 3 μ in diameter, each with a single cilium and an oil globule. Resting spores not observed.

Parasitic on *Melosira varians*, *Tabellaria* sp. (?), Fall Creek, Forest Home, Aug. 1931.

The writer is not entirely certain that he has assigned this fungus to the right species. The sporangia of Nowakowski's organism are more broadly "radish-shaped," possess an apiculate terminal portion, considered by him to be an operculum, as well as a lateral operculum, from which he assumed that the few zoöspores escaped (their emergence was not actually observed). The present fungus more nearly approximates *C. versatile* in the shape of its sporangium and in its terminal operculum which conforms to the contour of the sporangium. Further, the rather broad, basal stalk of the sporangium is intramatrical and not extramatrical as was the case in Nowakowski's fungus. Whether or not the sub-apical protuberance of the present fungus is really an incipient operculum cannot be determined, as no instances of its dehiscence were observed. It may be necessary in the future to segregate these two organisms.

An interesting feature with respect to the sporangial discharge of *C. epithemiae* as compared with that of *C. versatile* was observed. After disassociation of the spore mass in the former fungus, the cilia of the zoöspores appeared to be curled around the spore bodies and not, as in the latter species, directed toward the mouth of the sporangium (text fig. 1, *f*).

6. *Chytridium* sp. (?) (text fig. 1, *o*).

An empty sporangium, similar in shape to that of the preceding species, 18 μ in diameter by 25 μ in length, with a tapering, intramatrical portion and a lateral operculum about 6 μ in diameter, was found on a species of *Cladophora*. As no zoöspores were observed, the writer hesitates to assign the plant to any species and presents it here merely to call it to the attention of other investigators.

Parasitic on *Cladophora* sp. (?), Fall Creek, Forest Home, Oct. 1931.

7. **Chytridium curvatum** n. sp.

Sporangium extramatrical; smooth-walled; strongly arched; broadly pyriform or clavate; 8 μ, tapering to 5 μ in diameter, by about 18 μ in length; possessing at the base a thick-walled, goblet-like sterile portion from which a short, peg-like, hyaline stalk emerges; the latter not penetrating the host wall but merely attached to it; provided with an apical operculum about 5 μ in diameter (text fig. 1, *n*). Zoöspores uniciliate, 5 μ in diameter with a single oil globule; escaping upon the dehiscence of the operculum. Resting spores not observed.

Epiphytic on (?)*Stigeoclonium* sp., Fall Creek, Aug. 1931.

Sporangiis clavatis v. piriformibus, curvatis, operculatis, 8 $\times$ 18 μ; parte basali sterili praeditis; basi filamentis extramatricalibus brevibus munitis, in alga superficialibus; zoösporis globosis, 1-ciliatis, 5 μ diam., uniguttulatis; sporis perdurantibus ignotis.

Hab. in (?)*Stigeoclonium* sp., New York.

A number of examples of this interesting fungus were observed, all seemingly epiphytic on the alga. They were readily detached from the filaments of the host by lightly tapping the cover glass. No disintegration of the contents of infected host cells could be noticed.

Aside from its arched habit, the organism superficially resembles *Podochytrium clavatum* Pfitzer, especially in the possession of a sterile basal portion. However, the presence of an operculum, as well as the character and mode of attachment, sharply demarks it from *Podochytrium*, as well as from other species of *Chytridium*.

8. **Chytridium appressum** n. sp. (text fig. 1, *p*).

Sporangium extramatrical, smooth-walled, resembling a foot print in optical section, the long axis parallel with that of the diatom filament; with a highly refractive, cylindrical tube about 3 μ in length which penetrates the host wall, arising from the slightly expanded, basal portion; 10–14 μ in length by 6 μ at its maximum diameter; with an apical operculum 3–5 μ in diameter, which, upon dehiscence allows the fully formed zoöspores to escape. The latter bodies uniciliate, 3–5 μ in diameter, with a single oil globule. Resting spores not observed.

Parasitic on *Melosira varians*, Fall Creek, Forest Home, Aug. 1931.

Sporangiis similis pedibus in sectione, ad alga appressis, 10–14 μ $\times$ 6 μ, operculatis, basi filamentis brevibus simplicibus munitis; zoösporis globosis, 1-ciliatis, 3–5 μ diam., uniguttulatis; sporis perdurantibus ignotis.

Hab. in *Melosira varians*, New York.

This fungus was found to be fairly abundant on filaments of *Melosira*, the cell contents of which were greatly disorganized by the parasite. There were practically no variations in the horizontal position of the sporangia with reference to the long axis of the diatom filament or in the character of the highly refractive, unbranched penetration tubes.

While somewhat resembling the previous species, *C. appressum* lacks a sterile basal portion and, in contrast to the epiphytic habit, possesses an intramatrical tube. A somewhat similar appearing plant is figured by Dangeard (3, pl. 13, fig. 29*b*) in his account of *C. Schenkii*. However, the intramatrical swelling and the presence of rhizoids added to the obvious fact that the horizontal sporangium figured was atypical, would seem to mitigate against the two forms being the same.

9. *Nowakowskiella elegans* (Nowak.) Schröter, *l. c.*, p. 82.

> *Cladochytrium elegans* Nowakowski, *l. c.*, p. 95, Pl. 6, figs, 14–17.
>
> *Nowakowskiella endogena* Constantineanu, Rev. Gen. Bot. **13**: 397, fig. 83.

Mycelium intra- or extramatrical, well developed, profusely branched, the ultimate tips of the branches becoming rhizoidal in character; extremely variable in its diameter. Sporangia usually terminal, sometimes intercalary; generally apophysate; of various shapes, with or without a tube of discharge (rarely more than one); varying greatly in size, the majority being about 25–30 μ in diameter. Zoöspores 5–7 μ in diameter; uniciliate; spherical, with a single oil globule; fully formed within the sporangium; emerging upon the dehiscence of an operculum about 5–7 μ in diameter, and forming at the orifice of the sporangium a compact, spherical mass surrounded by a vesicular structure or "slime"; the latter soon bursting and liberating the spores.

Saprophytic in soil and cultivated on various seeds and bits of corn stem. Ithaca, Aug. 1931.

Nowakowskiella has been frequently encountered here and in New Hampshire growing as an extramatrical saprophyte among various saprolegniaceous forms, notably *Thraustotheca*. It was of interest to observe that when growing intramatrically, the shape of the sporangium and length of the discharge tube were frequently modified by the surrounding host tissue, although these structures were observed to vary even when the fungus was growing free in the water (text fig. 2, *a*).

Certain early stages in the discharge of the zoöspores were exhibited with great clarity in the present material. Upon the initiation of zoöspore discharge, the operculum is carried up for a short distance on the emerging, expanding, terminal hyaloplasm (text fig. 2, *b*, *c*), but shortly slips back near the orifice of the sporangium (text fig. 2, *d*). Shortly after the condition shown in figure 2, *c*, the zoöspores commence to emerge and the hyaline, apical material is lost from view. Whether or not this substance is the material from which is formed by continuous expansion an enveloping vesicle, could not be determined. It is possible, however, that this is what actually happens.

Occasionally, in old desiccated bits of grass, rather brown, thick-walled, spherical or elongate resting bodies are observed (text fig. 2, *f*, *g*). While these structures nearly always occur in the same stems as those in which

Nowakowskiella is growing, the writer has not demonstrated to his satisfaction that they are produced by this fungus.

A sporangiophore somewhat resembling that of *Tetrachytrium triceps* Sorok. was encountered among material of *Nowakowskiella* growing on submerged corn stems (text fig. 2, *e*). So far as could be ascertained, this struc-
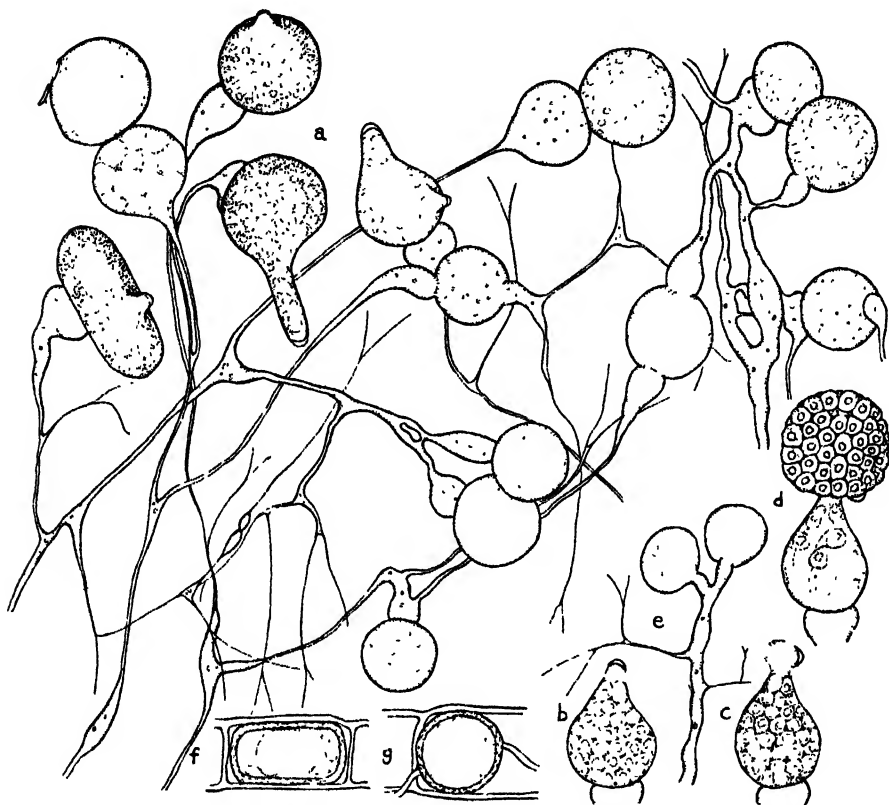
TEXT FIG. 2. *Nowakowskiella elegans. a*, Habit of plant growing as an extramatrical saprophyte, ×600; *b*, Mature sporangium about to discharge zoöspores, ×600; *c*, Emergence of apical hyaloplasm after dehiscence of operculum, ×600; *d*, Zoöspores nearly all emerged, forming a compact group at mouth of sporangium, ×600; *e*, Sporangiophore bearing two small, discharged sporangia. In habit, reminiscent of *Tetrachytrium*. ×600; *f, g*. Resting spores formed in cells of corn stalk. These bodies are frequently found when *Nowakowskiella* is growing within this host, ×600.

ture, which bore two sporangia, was organically related to the *Nowakowskiella*, although the motionless spores seemed somewhat smaller than those of the latter fungus.

Endochytrium n. gen.

Mycelium intramatrical; without swellings or turbinate cells; ultimate tips of branches rhizoidal. Sporangium intramatrical; discharging the fully formed, posteriorly uniciliate zoöspores after the dehiscence of an operculum.

Mycelio copioso, intramatricali, ramosissimo, rhizoidibus; sporangiis intramatricalibus, operculatis; zoösporis 1-ciliatis.

10. **E. ramosum** n. sp.

Mycelium intramatrical; extensive, irregularly and profusely branched, the ultimate tips rhizoidal; ramifying throughout the infected filament, not confined to a single cell; often expanded in the region of attachment to the sporangium; attaining a diameter of 10 μ in some instances. Sporangium ovate, nearly spherical, or sometimes pyriform, 35 μ in diameter, with a short, broad tube of discharge which just penetrates the host wall (pl. 2, *E*, *F*, *G*); with an apical operculum, 7 μ in diameter, which, upon the maturity of the zoöspores, dehisces and allows the latter to escape. Zoöspores uniciliate; spherical or somewhat elongate; 3–5 μ in diameter; with a single oil globule; upon escaping from the sporangium forming for a few moments an ellipsoidal, motionless mass a short distance above the orifice of the sporangium (pl. 2, *A, B, C, D*). Resting spores not observed.

Parasitic on *Cladophora* sp. (?), Bessemer, N. Y., Jan. 1932.

Characteribus generis. Sporangiis sphaeroideis v. pyriformibus, circa 35 μ diam., operculatis; zoösporis globoso-ellipsoideis, 3–5 μ diam., 1-ciliatis, uniguttulatis; sporis perdurantibus ignotis.

Hab. in *Cladophora* sp., New York.

In its general aspect, *E. ramosum* closely resembles certain species of *Entophlyctis*, particularly the " giant thalli " described recently on *Cladophora* by Karling (7). The radically different method of reproduction, however, seems to indicate that this superficial resemblance is in reality a case of convergence. Whether or not the pyriform sporangia shown in plate 2, figure *E*, are variations of the more numerous spherical ones, or whether they belong to another, distinct species of the genus, will perhaps be clarified by subsequent collections of this fungus.

The mycelium, which from the illustrations appears very evident, was in fact extremely difficult to observe. This was due not only to the presence of large amounts of disintegrating chloroplasts, but also to the peculiar refractivity of the mycelium. Another feature of the fungus was the persistence of the operculum. These bodies could be observed near the sporangial orifices several weeks after dehiscence.

Whether or not the fungus was truly parasitic was not determined with certainty. It was noted, however, that sporangia were usually found in algal cells, the contents of which were greatly disorganized.

Thus far, resting spores have not been found although a systematic examination of infected *Cladophora* cultures has been maintained for some months.

11. ?**Endochytrium oöphilum** n. sp.

Mycelium not observed. Sporangium intramatrical; ovate; with a short papilla which barely penetrates the cell wall of the egg and which terminates in an operculum about 6 μ in diameter (text fig. 1, *r*); 50 μ in length by

30–35 μ in diameter. Zoöspores formed within the sporangium; uniciliate, spherical or somewhat elongate; about 3 μ in diameter; with a single oil globule; liberated with great rapidity upon the dehiscence of the operculum (text fig. 1, s). Resting spores not observed.

Parasitic in Rotifer egg, Bessemer, N. Y., Jan. 1932.

Sporangiis intramatricalibus, globoso-ellipsoideis, operculatis, 30–35 × 50 μ; zoösporis ellipsoideis, 3 μ diam., 1-ciliatis, uniguttulatis; ceteris characteribus ignotis.

Hab. in ovis Rotiferorum, New York.

This fungus has been tentatively assigned to *Endochytrium* until more is known about its vegetative state. When the few infected eggs were first observed, sporangia had already been formed, and although a search was made for younger stages in the development of the fungus, they were not found. If, upon further investigation, it is found that the content of the zoöspore which had effected penetration of the egg develops directly into the sporangium without the production of vegetative hyphae or rhizoids, the advisability of retaining this form in *Endochytrium* may well be questioned. The possibility cannot be overlooked, however, that some sort of vegetative system even more tenuous than that found, for example, in *Catenaria* may have been present in this material but escaped detection. From previous experience with *Catenaria* (10) the writer was particularly careful regarding this point, but in the absence of material in earlier stages of development, no final disposition of the fungus can as yet be made.

The obvious resemblance of *E. oöphilum* to *Olpidium gregarium* Nowak., another parasite of Rotifer eggs, would seem to indicate that we have here another case of convergence and adaptation to a particular type of habitat by two unrelated fungi.

12. *Megachytrium Westonii* Sparrow, Occas. Papers Boston Soc. Nat. Hist. **8**: 9, 1931.

For convenience the following generic and specific diagnoses are included.

Megachytrium.

Mycelium extra- and intramatrical; profusely branched and extensive; variable in diameter but usually of great size, never rhizoidal; occasionally septate; possessing intercalary or terminal swellings, marked off by cross walls. Sporangium irregular in size and shape; intercalary or terminal; opening by an operculum. Zoöspores posteriorly uniciliate. Resting spores thick-walled, usually intercalary; intra- or extramatrical.

M. Westonii.[1]

Mycelium at first entirely extramatrical, later intramatrical; 5–7 μ in diameter, with smaller branches about 3 μ in diameter; with terminal or inter-

[1] Named in honor of an ardent student and stimulating teacher of the Phycomycetes, Prof. W. H. Weston, Jr., of Harvard University.

calary swellings; markedly undulating. Sporangium terminal or intercalary; sometimes spherical or clavate, but more often of irregular shape; with or without a discharge tube; varying greatly in size, usually about 15–50 μ in length by 10–30 μ in diameter; sometimes apophysate; rarely proliferating. Zoöspores fully formed within the sporangium; escaping upon the dehiscence of an operculum 3–5 μ in diameter; spherical, 5 μ in diameter, with a single small oil globule. Resting spores intercalary, intraor extramatrical; thick-walled, broadly ovoid with truncated ends; usually about 20 μ long by 15 μ in diameter; germinating by means of a sporangium formed outside the wall of the spore.

Parasitic on *Elodea canadensis*, Fall Creek, Forest Home, Oct. 1931.

This remarkable fungus caused a pronounced discoloration and disintegration of the leaves of *Elodea*. Due to the opaque character of the host some difficulty was encountered in tracing its development.

The zoöspore upon germinating produces a rather broad, undulating hypha which, as it grows over the host surface, expands and branches (pl. 3, *Ca*). The mycelium ultimately produced varies greatly in diameter, is markedly undulate, and shows in the younger stages of development a tendency to follow the region of juncture of the host cell walls (pl. 3, *A*). The latter fact would seem to suggest some type of pectin relationship. Further growth and branching are profuse (pl. 3, *B*). The hyphal contents are finely granular, refractive, with large vacuoles and are occasionally separated by narrow cross walls. The ultimate branches of the mycelium are extremely refractive and often fuse laterally with one another in a very characteristic manner (pl. 3, *C*). ' It is particularly noteworthy that, in contrast to other chytridiaceous forms possessing a well developed vegetative thallus, such as *Nowakowskiella*, the mycelium never becomes rhizoidal in character. In heavily parasitized leaves the mycelium is found to be intra- as well as extramatrical, although the method whereby the fungus gains entrance to the host has not as yet been ascertained. On the stouter portions of the mycelium large, broadly fusiform or irregular swellings delimited by cross walls are produced. These may form either sporangia or resting spores (pl. 3, *B*). The latter (pl. 3, *Da*) may germinate after little or no resting period. In this process the wall is partially assimilated and a sporangium is formed outside the wall of the spore (pl. 3, *Db*). Zoöspores are formed in the usual manner (pl. 3, *Dd*).

The sporangia originate as somewhat pyriform, terminal enlargements of the mycelium (pl. 3, *A*). When mature they are variable in shape and usually possess a slightly inflated apophysis (pl. 3, *B, Dc*). Proliferation has been noted in a few instances.

In erecting a new genus for the disposition of this fungus, the writer was not unmindful of the fact that two similar-appearing fungi had previously been described by Zopf. The first of these, *Hyphochytrium infestans* Zopf (13), was described in 1884 as a parasite of *Helotium*. The mycelia of

Megachytrium and *Hyphochytrium* are somewhat similar, both possessing elongate intercalary swellings and occasional septations, but lacking rhizoidal attenuations. In other respects they differ from one another. *Hyphochytrium* possesses a spherical sporangium with a prominent apical papilla; it opens by a sub-apical pore (not an operculum) and produces *anteriorly* uniciliated spores. In the latter respect the fungus differs from all valid members of the order. Even if based on correct observations, which seems unlikely, it appears distinct from the present fungus. Vuillemin (11) considers Zopf's organism to be a filamentous fungus attacked by a parasite, and is supported in this contention by von Minden (8) who places it in a new genus *Hyphophagus*.

In 1888 Zopf (15) described another fungus which shows a slight resemblance to *Megachytrium*. It was causing root rot of *Stiftia* and was termed *Protomyces radicicolus*. The rather broad mycelium (8–12 μ in diameter) was frequently septate, became brown with age, and produced haustoria which penetrated the host cells. Thick-walled, fusiform, or irregular intercalary bodies were formed. No other phases in the reproduction of the plant were observed. Zopf also proposed the name *Physotheca* for the fungus in case it should prove distinct from *Protomyces*. In its present imperfectly known state and in view of the fact that the writer has recently observed in similar habitats several Hyphomycetes which bear a close resemblance to *P. radicicolus*, he has preferred to consider the parasite of *Elodea* as wholly distinct from Zopf's organism.

DISCUSSION

It has been the writer's experience in working with chytridiaceous organisms, that forms liberating their spores after the dehiscence of an operculum *always* discharge them in this manner. For example, all the sporangia on a thallus of *Nowakowskiella* are operculate, and plants grown from zoöspores of this plant will form sporangia which, in turn, will be operculate. Similarly, a plant of *Cladochytrium* or *Rhizophidium* will form sporangia which will discharge their spores after the deliquescence of a papilla and these spores will form other plants, the sporangia of which will liberate their spores in a similar manner. There has not as yet been described a valid species of the group in which a single plant bears both operculate and inoperculate sporangia. However, the possibility of the occurrence in nature of such an organism cannot be overlooked. In a similar manner one might discuss *Entophlyctis* and *Endochytrium* or *Rhizophidium* and *Chytridium*.

When more data of unquestioned value are accumulated concerning the sexuality of the chytrids, it may be possible that a much more fundamental basis will be found for the classification of these organisms than that of the thallus. In the present state of our knowledge, however, the presence or absence of so definite a morphological structure as the operculum might well be considered in a more fundamental light than heretofore. It is possible

that such action may aid considerably in the establishment of some degree of phylogenetic order within the group.

One further point seems worthy of mention. It has become increasingly apparent to the present investigator that there exists an enormous aquatic and semi-aquatic microscopic flora, only a few scattered members of which have as yet been observed. Not only have these forms been sought in relatively few regions of the world, but when found they have been so modified by their habitat and mode of life and so prolific in examples of parallelism that their natural affinities are by no means readily apparent. In view of these facts, it seems to the present investigator, at least, that any extensive discussion of the phylogeny and interrelationship of the chytrids at this time is more than futile.

However, one idea of possible phylogenetic significance is suggested by the organisms described in this paper; namely, that certain operculate forms have proceeded along a line of thallus development characterized by the production of rhizoids (*C. versatile, C. sphaerocarpum, C. Schenkii, Endochytrium ramosum* and *Nowakowskiella*), whereas others are seemingly devoid of such structures (*C. epithemiae, C. appressum, C. curvatum* and *Megachytrium*). Just how important this suggestion really is must await the time when our knowledge of these organisms is considerably enhanced.

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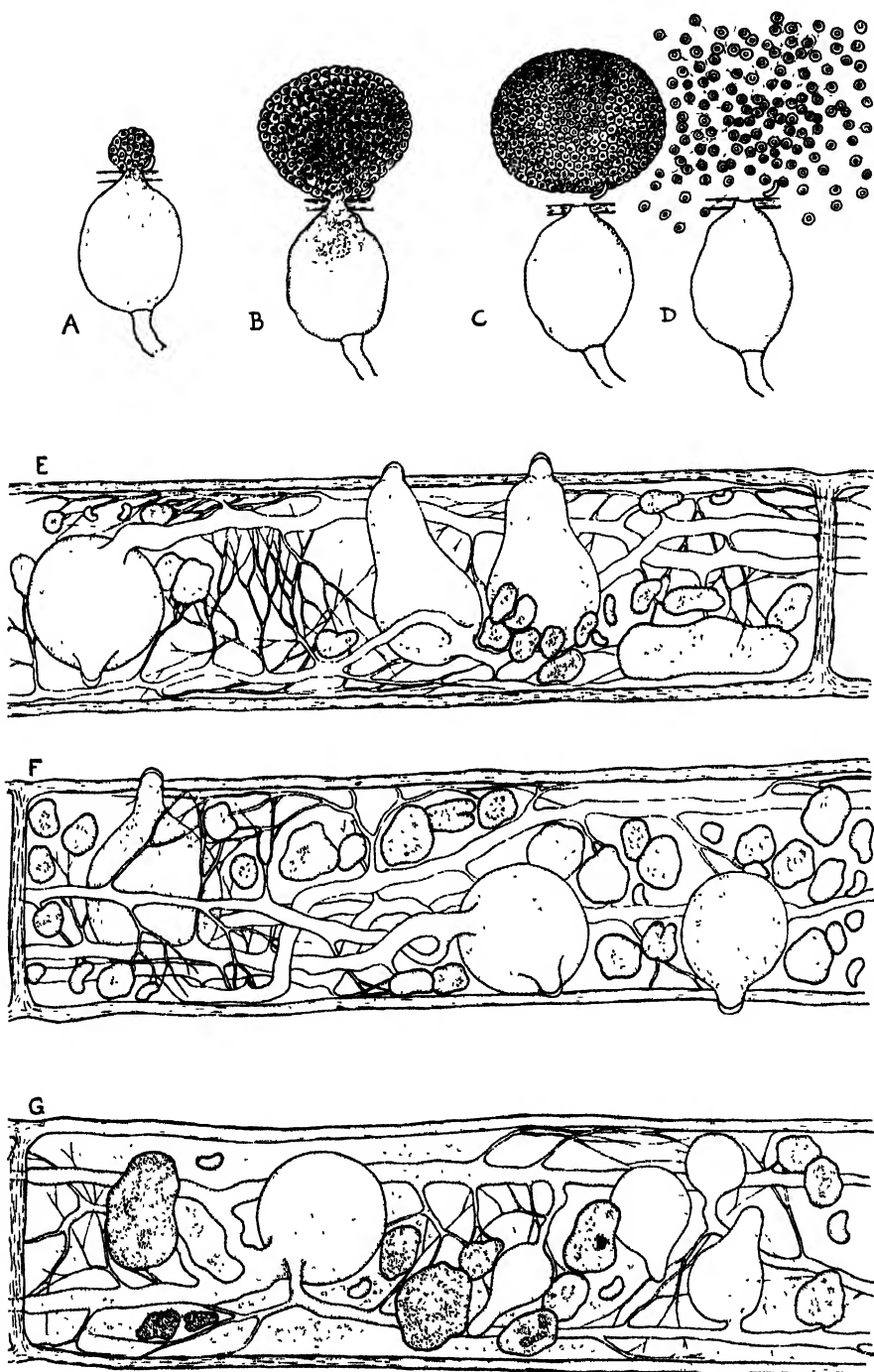
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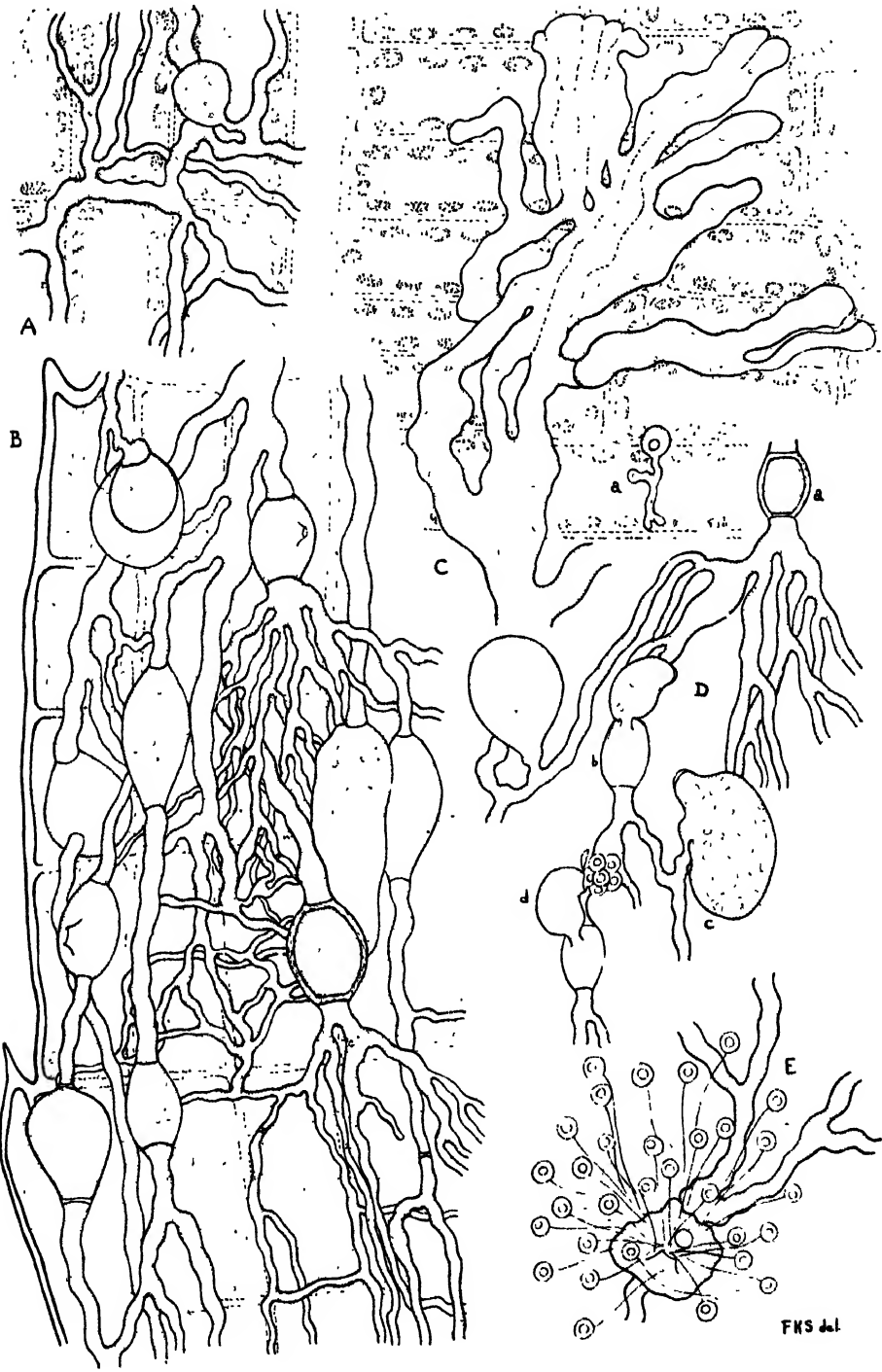
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EXPLANATION OF PLATES

All figures were drawn from living material with the aid of the camera-lucida. The approximate magnifications are given in each instance.

PLATE 2

Endochytrium ramosum n. g.; n. sp.

FIGS. *A-D*. Stages in the liberation of the zoöspores. FIG. *A*, dehiscence of operculum and initiation of discharge of coherent spore mass. FIG. *B*, spore mass almost completely discharged (optical section). FIG. *C*, spore mass completely discharged and remaining as a motionless mass near orifice of sporangium. FIG. *D*, disassociation of spore mass and dispersal of zoöspores. Only a few of the latter bodies are shown. All × 420.

FIG. *E*. Habit of fungus in a cell of *Cladophora* (combined views), showing pyriform as well as more typical spherical sporangia. × 420.

FIG. *F*. Another infected cell (combined views) showing sporangia of more typical shape. × 420.

FIG. *G*. Similar habit showing younger stages in the development of the sporangia (combined views). The great breadth the mycelium may attain at the point of attachment to the sporangium is well illustrated here. × 420.

PLATE 3

Megachytrium Westonii

FIG. *A*. Early stage in the development of the mycelium and a sporangium. No noticeable effect of the fungus on the *Elodea* leaf is as yet observed. × 420.

FIG. *B*. Portion of a leaf of *Elodea* showing a late stage in the development of the fungus which is characterized by a profuse mycelial growth with terminal or intercalary hyphal swellings. The latter develop either into thick-walled resting spores or sporangia. × 680.

FIG. *C*. Highly refractive, ultimate branches of the mycelium showing their non-rhizoidal character and peculiar anastomses. *a*, germinating zoospore. × 680.

FIG. *D*. Portion of a thallus bearing (*a*) resting spore, (*b*) germinating resting spore, (*c*) sporangium, (*d*) discharging sporangium formed from resting spore. × 420.

FIG. *E*. Discharge of zoospores. The operculum may be seen as a circular structure near the orifice of the sporangium. × 420.

ATTEMPTS TO DEMONSTRATE SYMBIOTIC NITROGEN-FIXING BACTERIA WITHIN THE TISSUES OF *CASSIA TORA*[1]

Ethel K. Allen and O. N. Allen

INTRODUCTION

From the standpoint of soil fertility the leguminous plants have for a long time maintained an exceptional position because of their symbiotic relationship with nitrogen-fixing bacteria. This symbiosis between plant and microörganism is commonly recognized by the visible manifestation of nodules on the root systems of these plants. Members of the sub-family Papilionatae are among the most common examples. Fewer data on the presence of nodules in the sub-families Mimosoideae and Caesalpinioideae are available, probably because their members are trees, shrubs or plants distinctly unsuitable as field crops. Of twenty-four members of the Mimosoideae cited by Leonard (9) only one, *Acacia Baileyana* F. Muel., lacked nodules, whereas twenty members of the Caesalpinioideae, also cited by Leonard, were found by various investigators to lack nodules. *Chamaechrista nictitans* (L.) Moench., *Chamaechrista fasciculata* (Michx.) Greene and *Chamaechrista Simpsoni* Pollard were exceptions.

It has often been suggested that the absence of nodules on various leguminous plants does not necessarily preclude the presence of nitrogen-fixing bacteria within the root tissue. For several reasons this question has never been thoroughly investigated. Joshi (7) was probably the first to suggest that root nodule bacteria existed in the root tissues of certain papilionaceous plants although root nodules were not formed. He proposed this explanation for the substantial increases in yield and high nitrogen contents which were obtained in the culture of these plants. Joshi further conjectured that such a condition might occur due either to a lower virulence of the specific organism or to a slower reaction of the plant to the stimulus of the organismal attack. His histological studies failed to show the presence of any organisms in the root tissues. It is now obvious that some of his results can be explained on a cross-inoculation basis. Feher and Bokor (5) and Friesner (6) have also proposed the idea that root nodule bacteria might benefit plant growth without forming nodules.

Critical examination of the above papers is difficult since the techniques were not satisfactorily described. On the basis of Joshi's paper one might

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explain the negative results on any of the following reasons: first, the organisms were not present in the root tissue; second, the organisms did gain entrance to the plant tissue and were established in localized areas, but the histological sections showing such a condition were overlooked in the examination; or third, the organisms were not demonstrable by the methods employed. Since the procedures for staining bacteria in tissue are comparatively recent, and since Joshi did not state his technique, one might assume that he employed some method suitable for ordinary histological procedure but which we know now to be wholly unsatisfactory for the differentiation of tissue and organism.

Several members of the sub-families Mimosoideae and Caesalpinioideae are used as green manuring crops under tropical conditions regardless of the absence of nodules and the apparent inability of such plants to fix atmospheric nitrogen. Van Helten (19), Allan (1), and Rant (17) have reported favorable results in crop rotation by using *Cassia occidentalis* L. and *Cassia Tora* L. Even though nodules have been reported on *C. occidentalis* it is to be considered extremely uncommon (10). *C. Tora* has never been known to bear nodules. In a more recent study involving various nodulated and non-nodulated leguminous plants Leonard and Reed (10) found that *C. Tora* not only gave the greatest yield of hay but that no ill effects were noted in the subsequent indicator crops. These studies were conducted at the green manuring experiment station at McNeill, Mississippi.

Recent interest in *C. Tora* as a possible intercycle crop in pineapple culture gave impetus to the present attempts to demonstrate microörganisms existing within the plant tissues.

METHODS

C. Tora plants growing under natural field conditions as well as plants growing in the greenhouse in various combinations of culture were used. The material furnished by the greenhouse culture included: plants grown from sterilized seed in sterile quartz sand, plants grown from unsterilized seed in sterile quartz sand, and plants grown in sterile quartz sand from sterilized seed inoculated with soil from vigorous *C. Tora* plants occurring in the field. The seeds were "sterilized" or freed of surface microörganisms by immersion in a 1:1000 solution of corrosive sublimate for three minutes, followed by immersion in sterile hot water (65° C.) for two minutes. They were then rinsed four times in cool sterile distilled water. These plants were given Crone's nutrient solution once a week and sterile distilled water as often as necessary. The plants cultured under natural field conditions were grown from untreated seeds. Histological sections were made from young seedlings, plants of one month's growth, two months' growth, and mature plants.

Various histological methods for the staining of bacteria within the tissues were employed. Owing to the general skeptical attitude towards the methods and techniques concerning fixatives for bacteria in tissues, the

procedures were first tried on nodule tissue of *Cajanus Cajan* (L.) Millsp. and *Crotalaria anagyroides* H.B.K. The methods that proved satisfactory in staining bacteria in the nodule tissue were then used on the *C. Tora* material.

The fixatives used were those of Zenker, Altmann, Flemming, Benda, Champy-Kull, Murray and Wallin. For such of the fixatives as were unsuitable for bacterial staining because of the presence of acid, the modified formulae recommended for bacterial staining were used. The following staining methods were employed in combination with the above fixatives: Altmann's acid fuchsin, Flemming's triple stain, Heidenhain's haematoxylin, Champy-Kull's technique for mitochondria and bacteria, the Brown and Breen method for the differentiation of gram-positive and gram-negative tissues, the Kopeloff and Beerman modification of the Gram stain, Wallin's anilin-fuchsin-methyl-green stain for differentiating mitochondria and bacteria, and the MacCallum stain for demonstrating influenza bacilli in tissue.

In our first studies nodules of *Crotalaria anagyroides*, *Cajanus Cajan*, and the *C. Tora* material were passed through the ethyl alcohol and xylol series prior to embedding in paraffin. Owing to the fact that the tissue of *C. Tora* proved to be of a brittle woody nature and therefore split easily in sectioning, the recently developed normal-butyl-alcohol method of Zirkle (22) was tried on some of the material. By this method thinner sections were obtained than would have been possible by the ordinary dehydration procedures.

It was deemed necessary to section the principal parts of the *C. Tora* plants since several investigators have reported that in various non-leguminous species microörganisms seem to function in a symbiotic relationship with various tissues. Koorders' work (8) was probably the first to indicate this possibility. He observed microörganisms in the hydathodes of the flower buds of various tropical plants. Zimmermann (21) reported the occurrence of bacteria in nodules on leaves of *Pavetta indica* L., *P. lanceolata* Eckl., *P. angustifolia* Thw., and *Grumelia mikrantha* Hiern. of the family Rubiaceae. To this same family Boas (2) added two more examples in the genus *Psychotria*. Von Faber (3, 4) confirmed the results of Koorders, Boas, and Zimmermann and in addition showed somewhat conclusively that the microörganisms in the plant tissues were inherent symbionts accompanying the entire life cycle of the plants. Miehe (13, 14, 15) showed the same relationship to exist for *Ardisia crispa* A. DC. in the family Myrsinaceae.

Plants of *Pavetta indica* L. var. *tomentosa* (Roxb.) Hook. f. with and without leaf nodules were noted by von Faber (3). A study of these plants lacking nodules revealed organisms at the vegetative tips of the plants and in the seeds. He proposed a possibility of symbiotic relationship of an inherent nature which was confined only to the seeds and to growing tips but not to the leaves. Better growth occurred, however, when the nodules were conspicuous on the leaves.

In our studies roots, stems, flower buds, pistils, leaves, and seeds of *C. Tora* were sectioned. Both longitudinal and cross sections in series were made when it was considered necessary for more accurate examination.

RESULTS

Microörganisms were not demonstrated in any of the tissues of *C. Tora* regardless of the methods of plant culture, or of histological preparation or of methods of sectioning. On the basis of such results it seems logical to conclude that *C. Tora* does not contain within its tissues any microörganisms which serve in the role of nitrogen fixation. In the light of the recent work of Starkey (18) it is probable that *C. Tora* as well as other non-nodulated leguminous plants exert some stimulating influence on particular types of the bacterial flora of the soil such as the non-symbiotic nitrogen-fixing bacteria. This might partly explain the beneficial results obtained with these plants in field cultivation.
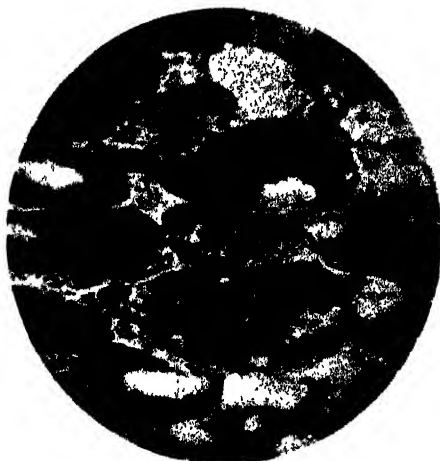
TEXT FIG. 1. Photomicrograph of bacteria in the tissue of a nodule from *Cajanus Cajan*. Murray's fixative followed by MacCallum's stain. × 1250. (Photomicrograph by D. M. Weller.)

Bacteria were demonstrated in the nodule tissues of *Cajanus Cajan* and *Crotalaria anagyroides* by all the staining techniques employed. In general it was found that longitudinal sections were more satisfactory for accurate examination than were the cross sections. The Kopeloff and Beerman modification of the Gram stain gave the best results when the time of staining was increased as suggested by McCoy (12). Murray's (16) and Wallin's (20) fixatives followed by MacCallum's (11) stain gave the most satisfactory results on the nodule material. These techniques afforded excellent differentiation of nodule tissue cells as well as distinct definition of bacterial cells (text fig. 1). Heidenhain's haematoxylin and Flemming's triple stains gave the least satisfactory results regardless of the fixative used.

Zirkle's normal-butyl-alcohol method for the dehydration of woody tissue was found to be highly satisfactory in the sectioning of *C. Tora* material.

SUMMARY

1. *C. Tora* is a leguminous plant commonly recommended as a green manuring crop regardless of its lack of nodules. It was attempted in these studies to demonstrate microörganisms within its tissues. Such a demonstration would be helpful in explaining the beneficial results obtained with it as a rotation crop.

2. Roots, stems, flower buds, pistils, leaves and seeds of *C. Tora* grown in various combinations of culture under greenhouse and field conditions were examined histologically.

3. Seven fixatives in combination with eight methods of staining were used in this study.

4. Microörganisms were not demonstrated in any of the tissues examined.

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SHRINKAGE AND EXPANSION IN WOODY CYLINDERS OF LIVING TREES

Ferdinand W. Haasis

It was somewhat over a decade ago that Doctor D. T. MacDougal of the Carnegie Institution developed an instrument for procuring continuous records of changes in diameter occurring in tree trunks. Among the facts made evident by the use of this device is that under certain conditions boles of living trees may be expected to show reduction in diameter as well as increase. Most studies of reversible variations of this sort have had to do with fluctuations in the diameter of the entire bole including a thin layer of bark (3, p. 20-21). A few investigations have, however, been made of such changes in the woody cylinder of Monterey pine trees (*Pinus radiata* Don.) and the results of preliminary studies of this kind were reported by MacDougal in 1921 and 1924 (2; 3, p. 39–40; 6, p. 41–42). The present paper presents the results of additional investigations along these lines.

While MacDougal has described in detail the instrument used in these studies, the dendrograph (3, p. 10--15), a brief delineation of its essential features may not be out of place here. The fundamental elements of the dendrograph are two contact rods borne on a rigid metal frame which is placed about the tree to be studied. This frame is built up of rods or bars of steel having a low coefficient of expansion, such as Invar, Bario, Permant, or Stoic. One of the contact rods is clamped rigidly to the frame and seated against one side of the tree. The other rod is seated against the tree on the opposite side, but is free to move in the direction of the diameter which is to be measured. The outer end of this movable rod bears against the short arm of an L-shaped lever. To the longer arm of this lever there is attached a pen whereby a graph can be traced on a record slip fastened about a cylinder. The cylinder is turned by clockwork, one revolution a week. By virtue of the relative lengths of the two lever arms, the record of variation in diameter may be magnified conveniently 10, 20, or as much as 30 times. The metal frame is suitably supported in position by light wires fastened to a belt of blocks clamped about the tree. The clock also is customarily fastened to this belt. In text figure 1 there is pictured a tree to which are attached three of these dendrographs. It is the usual practice in installing such an instrument to pare the bark down to a thickness of 1 or 2 mm. at the contact points.

The method used in the above-mentioned investigations of expansion and contraction in the wood (2, 3) was to study, first, reversible variations
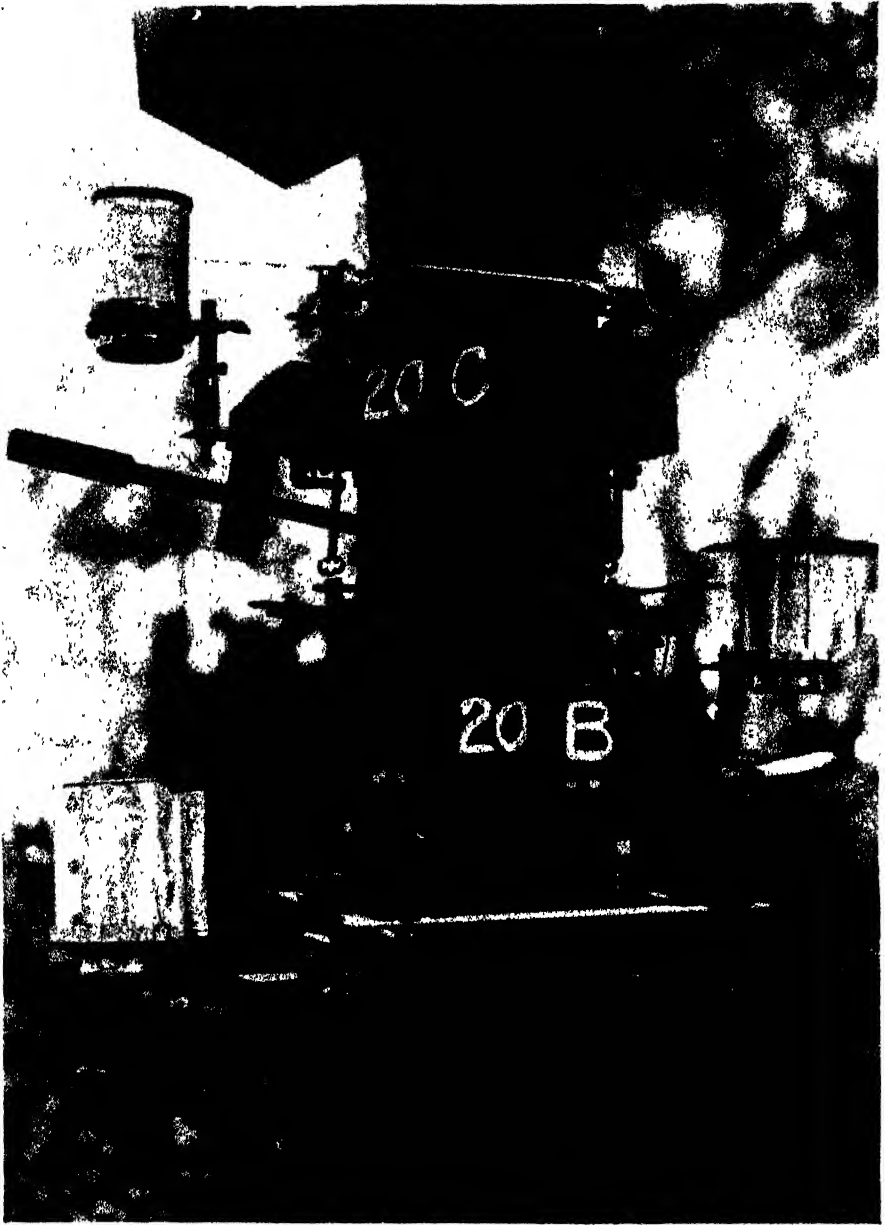
TEXT FIG. 1.   Tree of Monterey pine, 25 cm. in diameter, carrying three dendrographs. The contact rods of the lowermost instrument are seated on a thin layer of smoothed bark; those of the uppermost on recently formed wood, and those of the middle dendrograph at the bottoms of small holes, each extending into the tree a distance equal to one-fourth the diameter. The fixed contact rod can be seen on the left side of the middle instrument, the movable rod at the left side of the lowest instrument. The lever and cylinder show best in the uppermost instrument. The floating frame for the lowermost instrument is made of four members, that for each of the other two of six members. Above the recorder of the uppermost instrument can be seen part of a hinged metal cover for protection from rain.

in the tree by means of an ordinary dendrographic setting. After this, the bark and a thin layer of wood were removed, and the variations studied in the wood alone, nearby trees being used as controls. MacDougal found that there is a considerable amount of diurnal fluctuation in diameter of the woody cylinders of living trees, although this is relatively somewhat less than the diametral changes of the tree as a whole. The greatest amount of fluctuation reported by MacDougal in the woody cylinder was 1 part in 1550, or 0.0006 of the distance between contacts.

As an extension of this type of study, a second dendrograph was attached early in 1930 to a tree already carrying one with the contact rods seated on a thin layer of bark, in the customary way. The contact rods of this second instrument penetrated through small holes about 5 cm. deep to the inner part of the 24-cm. bole, thus bearing on a cylinder having about half the diameter of the tree. In 1931 a third instrument with contacts on wood about 1 year old was added (text fig. 1). For this tree there are accordingly available, for a period of several months, three parallel sets of records of diametral change, one for the wood with its surrounding cambium and a thin layer of external tissues, one for a nearly entire woody cylinder, and one for an inner cylinder, here referred to as the *central woody cylinder*, or, briefly, the *central cylinder*.

The first fact which stands out in these records is that diurnal fluctuations in diameter occur even in the central cylinder of this tree, essentially paralleling those in the larger cylinder and in the tree as a whole. While these variations of the central cylinder are prevailingly smaller than those of the entire tree, yet they are quite definite, amounting to as much as 1 part in 1300, or 0.0008 of the distance between contact points. The maximal diurnal fluctuation in the entire tree in the year 1931 was 1 part in 460 (*i.e.*, 0.0022 of the diameter).

The central cylinder seems to be especially sensitive to changes in wind velocity. The evidence, however, is not sufficient fully to establish this as a fact. On the other hand, fog does not appear to affect diametral changes in the central cylinder very promptly, although it usually results in a reduction of the amount of shrinkage in an entire tree, as compared with that occurring in clear weather.

It is to be remembered that the larger woody cylinder, as studied in this tree, includes those layers of recently formed wood in which the movement of solutions mainly takes place (6, p. 13). In view of this fact it might well be anticipated that diurnal variations in this cylinder would be found to be relatively greater than in the central cylinder. As it happens, however, the greatest relative daily variation, 1 : 1900 (= 0.0005 of the distance between contact points), was somewhat less than the greatest in the central cylinder, 1 : 1300 (0.0008). And while early in the year these fluctuations were in general greater in the larger woody cylinder than in the smaller, yet later in the season the condition was reversed. Examples

of these relations are given in table I, in which the coefficients for the entire tree are added for comparison.

TABLE I. *Relative Diurnal Shrinkage in Diameter in Different Parts of a Monterey Pine Tree, at Various Times of Year; Expressed as a Fraction of the Diameter*

Date, 1931–1932	Entire Tree (Including 1 or 2 mm. of Bark on Each Side)	Woody Cylinder (Except for 1 or 2 mm. of Outer Wood on Each Side)	Central Cylinder (½ Diameter of Preceding)
Apr. 14, 15	0.0014	0.0004	0.0002
May 6	.0012	.0004	.0002
7	.0022	.0005	.0004
June 5, 6	.0014	.0004	.0008
July 8	.0012	.0004	.0006
9	.0010	.0004	.0006
Aug. 8	.0008	.0003	.0004
9	.0006	.0001	.0004
Sept. 8	.0008	.0003	.0004
9	.0010	.0003	.0004
Oct. 8	.0006	.0003	.0006
9	.0008	.0001	.0004
Nov. 7	.0006	.0001	.0002
8	.0008	.0001	.0004
Dec. 8	.0008	.0003	.0002
9	.0010	.0003	.0004
Jan. 4, 5	.0010	.0003	.0004

The second outstanding fact yielded by the records is that seasonal decreases in diameter may take place in the central cylinder while progressive increase in diameter is occurring in the tree as a whole.[1] For example, during the period from April 28 to May 19, 1930, the inner part of this tree shrunk 0.2 mm. while the records for the tree as a whole showed an increase of 1.8 mm. It may even happen that the central cylinder will enlarge while the tree as a whole is shrinking, as is shown by the following figures where the plus sign indicates increase in diameter and the minus sign decrease. Seasonal variation in this respect is also evident:

	Entire Tree	Central Cylinder
Sept. 21—Oct. 19, 1931	− 0.14 mm.	+ 0.27 mm.
March 2—June 29, 1931	+ 6.50 mm.	− 0.57 mm.

From these figures it appears that a Monterey pine tree may shrink in one region of the trunk while expanding in another, a meter or so higher or lower. Such relations are shown graphically in text figure 2. Whether these differences are connected with setting up of the stresses which result in the defect known as *ring shake* is a question that can not at present be answered.

[1] For a more extensive discourse on *seasonal shrinkage*, the reader is referred to a paper of the author's recently published elsewhere (1).

At other times both the central cylinder and the tree as a whole may be increasing simultaneously. During the period from June 16 to July 7, 1930, the entire tree increased 1.18 mm., while the central cylinder increased 0.36 mm. Irrigation (together with a small amount of rain) in November, 1930, at a time when weekly diametral increases of the entire stem had ceased, apparently resulted in renewal of the weekly increments. The central cylinder, on the other hand, which had been shrinking rather steadily since early in October, responded more tardily, and the shrinkage again began after a few weeks.
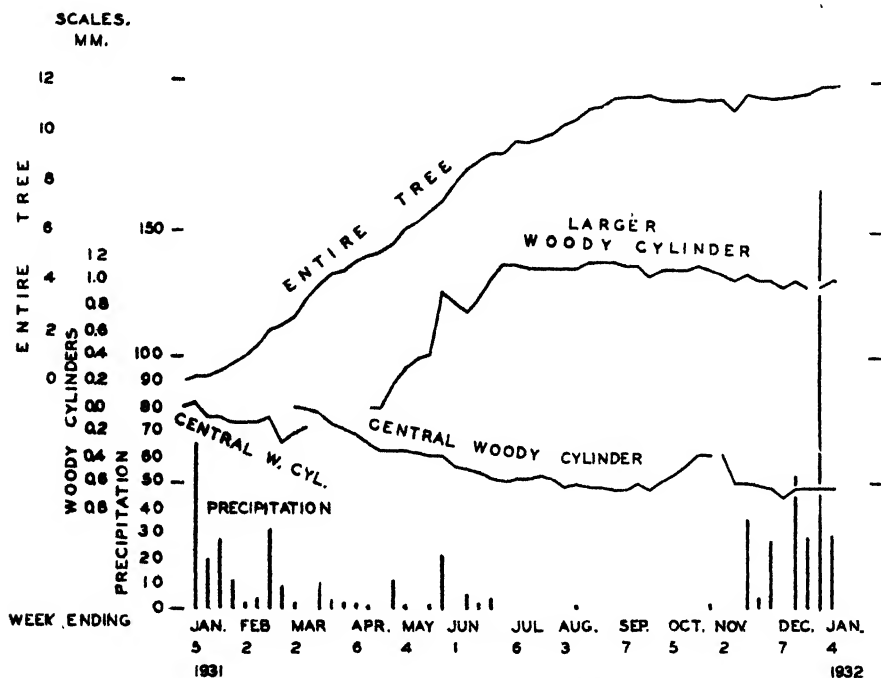
TEXT FIG. 2. Diametral changes, week by week, recorded by three dendrographs on a Monterey pine tree. The graph labeled "Entire Tree" is based on records obtained with a dendrograph whose contacts were seated on thin layers of smoothed bark; that labeled "Larger Woody Cylinder" on records made with contacts on recently formed wood; and those designated "Central Woody Cylinder" on records for a cylinder having half the diameter of the tree. For the last mentioned, a new instrument was installed in March, 1931. In each case the distance between contacts was plotted as zero for the beginning of the graph. Gaps in the graphs indicate poor records. Precipitation figures are added to show the relation of the diametral changes to rainfall. No explanation is at present forthcoming for the astonishingly large increase shown by the larger woody cylinder for one week in May.

The reasons for these apparent inconsistencies are presumably to be sought in the conditions obtaining in the hydrostatic-pneumatic system of the tree. There are always taking place in this system changes in tension brought about by varying intensities of a number of external and internal

8

'factors, such as air temperature, air humidity, and respiration (see Mac-Dougal and others (5, p. 10)). It is obviously not to be supposed that the effects of these changes will become evident at once in all parts of the bole.

Broadly considered, the program of shrinkage and swelling in the several parts of this tree is much the same. That is, the diameters of the two woody cylinders generally reach a maximum about the end of the period of darkness, and a minimum somewhat later in the day, as does the entire tree. The exact time at which the maximum or the minimum is attained, however, shows a considerable variability. Sometimes this may be the same for all three. Again, there may be a difference of many hours. For a few days in the middle of May, for instance, the woody cylinders reached their minimum 4 or 5 hours before the entire tree. Later in the month, on the other hand, the minima for the larger cylinder and the entire tree were at essentially the same time, whereas that for the central cylinder occurred as much as 7 hours earlier.

It is unnecessary to multiply examples. Suffice it to say that the divergencies are so irregular that the reasons underlying them are by no means evident. Presumably they are to be related to the variations in tension in the hydrostatic-pneumatic system mentioned above. It may be recalled in this connection that the program of expansion and contraction in the root of a Monterey pine is sometimes quite different from that in the bole (4, p. 35–37). It seems very likely that the explanation for these latter differences is the same as for those in the several parts of the trunk.

A dendrograph with contacts on an 11-cm. central cylinder of a 23-cm. redwood (*Sequoia sempervirens* Endl.) recorded a total shrinkage of 0.17 mm. during the period extending from June 8 to July 20, 1931. In the same period of time another dendrograph on the tree recorded a net shrinkage of 0.5 mm. for the entire tree. On the other hand, during the fortnight of September 7–21, when the tree shrunk 0.4 mm., the central cylinder increased 0.04 mm. in diameter. Daily variation as recorded by these two instruments during the period June 1, 1931–Jan. 11, 1932, amounted to maxima of 1 : 560 (0.0018) for the entire tree and only 1 : 2300 (0.0004) for the central cylinder.

Late in the fall of 1931, a dendrograph was fitted to a tree of coast live oak (*Quercus agrifolia* Neé.) with the contacts on the outside of the thick bark which is characteristic of this species. Another instrument attached to the same tree has its contact rods running into shallow bores so that they rest on wood about 2 or 3 years old. During the few months following installation the first instrument has recorded weekly fluctuations of as much as 0.48 mm., sometimes increases, sometimes decreases, but with a net increase in 22 weeks of 1.63 mm. The woody cylinder, on the other hand, has shown practically no increase, except during the first week, and at the end of 22 weeks had a diameter of 0.56 mm. less than at the beginning.

SUMMARY

Studies with the dendrograph, an instrument already described by D. T. MacDougal, have shown that:

1. Diurnal fluctuations in diameter may occur in the central part of a Monterey pine tree, that is, in a woody cylinder having half the diameter of the tree.

2. Seasonal decrease in diameter may take place in this small cylinder while progressive increase in diameter is occurring in the tree as a whole or, conversely, the central cylinder may show enlargement while the tree as a whole is shrinking: that is, one part of the bole may be shrinking while another is expanding.

3. The program of diametral shrinkage and swelling is in general similar for the central woody cylinder and for the entire tree, but with marked abnormalities as to time, presumably ascribable to variations in tension in the hydrostatic-pneumatic system.

4. Somewhat similar conditions appear to obtain in redwood trees.

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THE COLONIZATION OF THE KATMAI ASH, A NEW AND INORGANIC "SOIL"

ROBERT F. GRIGGS

The revegetation of any denuded area is of interest, but the colonization of volcanic ash offers an opportunity not to be had elsewhere for studying one of the most obscure and important processes of nature—the origin of organic nitrogen. For the colonization of the inorganic, nitrogen-free ash necessarily involves the initiation of the nitrogen "cycle." When, therefore, it became clear that the ash thrown out of Katmai Volcano in 1912 was exceptional in amount, studies of this problem were undertaken by the National Geographic Society. But during the earlier years the ash remained bare, and all that could be done was to lay the foundations for intelligently dealing with the problem when revegetation should begin. In 1930, however, eighteen years after the eruption, colonization had occurred, and by a group of plants, which, to the writer at least, would never have been suspected of ability to play the role of pioneers under the rigorous conditions obtaining—by leafy liverworts of the family Jungermanniaceae. Large areas of ash were overgrown by thick, felted carpets of liverworts, practically to the exclusion of all other plants. This paper is an account of the colonization of the ash by liverworts, and a discussion of the physical and biological factors involved.

Physically, finely divided volcanic ash is analogous to coarse sand or fine gravel and so offers a suitable medium for the penetration of plant roots. Chemically, the ash of Katmai corresponds to granite, and so probably contains all the minerals required for plant life though both potassium and phosphorous are deficient in quantity. But organically, having been produced from a molten magma extruded from the interior of the earth, it was at the outset entirely devoid of humus or of the microörganisms which are essential features of soils.

In the terminology of soil science, such a substratum does not constitute a "soil" at all, but is merely a mass of "soil material." The process of revegetation involves, then, not only the origin of organic nitrogen, but the genesis of a soil. Extensive fields of volcanic ash are, moreover, the only places where the beginnings of the process of soil formation can be observed without complications due to the inevitable organic contaminations brought in from old soils.

On the normal face of the earth are various situations ordinarily considered sterile habitats: new subsoil exposed by erosion, bare rocks where

plants can get hold only in the crevices, sand dunes washed up by the sea. But these habitats are only relatively devoid of organic matter. Actually they are all appreciably contaminated with it. The subsoil has been bathed for centuries with ground water percolating down from the plant cover above. Rock crevices catch much wind-blown debris. Even sea-sand includes the buried remains of marine animals and plants of all sizes from plankton to whales; and the sea water which threw it up contains ammonia in varying amounts, sometimes exceeding 1 part per million (see literature cited by Clark 1920, p. 119; also Seiwell 1931). In contrast to all other habitats, fresh volcanic ash is absolutely without organic contamination.

It might be supposed *a priori* that the organic matter in the most sterile of ordinary habitats such as beach sand would be so small in amount as to be negligible. This, however, is not at all the case. In the conditions they offer for plant growth, there are great differences between volcanic ash and the purest of sea sand. On the sand, if it be stable, plants of many species can grow with ease. But in volcanic ash, pure as it originally fell and without subsequent contamination, ordinary plants cannot grow. The admixture of a very slight amount of organic matter, may, as will appear, be of critical importance for the growth of plants. Secondary ash deposits laid down by wind or water and inevitably somewhat contaminated with organic matter are altogether different habitats than the original stratified ash lying undisturbed as it fell from the volcano.

Nowhere, then, except after a very great eruption which has deeply covered a large area with ash, is opportunity afforded for studying the colonization of an inorganic substratum on a large scale. In a lesser eruption or wherever the mantle of ash is less than two or three feet thick, surviving plants rooted in the old soil send their shoots through the ash, thus at once introducing organic contamination and so removing the area from the field of study. Again, except where a very great quantity of ash has been deposited, the forces of erosion quickly modify the ash fields by removal and redeposition, until after a few years little undisturbed ash remains. There is thus no opportunity for a study of colonization on the slopes of the volcano or in mountains anywhere, for it is only on a plain where the forces of erosion can take no hold that ground of the necessary stability occurs.

Examination of the literature of volcanoes will show that eruptions providing the conditions requisite for an extensive study of recolonization of uncontaminated ash are rare. Indeed, since the dawn of scientific interest in this matter, only two opportunities for such studies have occurred —Krakatau in 1883 and Katmai in 1912.

Credit for the realization that Katmai offered an opportunity duplicated only once in a generation, and for the initiation of steps to take advantage of that opportunity is due primarily to one man—Dr. Frederick V. Coville.

His vision of its importance was transmitted to the officers of the National Geographic Society, and that organization undertook the project and have carried on through fifteen years, sending six expeditions in charge of the writer into the region. To Dr. Coville, now chairman of the Research Committee and of the Board of Trustees, and to the President and Vice President of the National Geographic Society, Dr. Gilbert Grosvenor and Dr. John Oliver La Gorce, the writer owes a heavy debt for the constant support and encouragement which has made this work possible.

After the return from Alaska, I was fortunate in having the assistance of specialists working in the fields touched by the results. Dr. Alexander W. Evans of Yale has kindly identified the liverworts for me. Mr. J. E. Benedict of the George Washington University has identified the mosses. Dr. Franklin E. Allison and his associates of the Fixed Nitrogen Laboratory have given counsel and suggestions. The staff of the office of Soil Micro-biology of the Department of Agriculture, Dr. Charles Thom, Dr. N. R. Smith and Mr. Daniel Ready, have very generously assisted in many ways, furnishing chemical and bacteriological analyses of the materials as well as valued counsel. The coöperation of these gentlemen has greatly increased the thoroughness and completeness with which the work could be done, and has added especially to the effectiveness of the cultural studies which are now under way.

RESULTS OF THE EARLIER KATMAI EXPEDITIONS

During the years covered by the earlier expeditions of 1915, 1916, 1917, 1918, and 1919, while the ash remained barren, all that could be done with our primary project was to clear away the preliminaries required for an approach to the problem.

Meanwhile, we discovered the Valley of Ten Thousand Smokes, and through the coöperation of the Geophysical Laboratory of the Carnegie Institution made important contributions to a study of volcanism. Out of the earlier expeditions also have come a number of papers bearing on various aspects of the revegetation problem. The starting point for studies of this sort, the effect of the eruption on plant life, has been described by Martin (1913), by Rigg (1914), and by Griggs (1915, 1919 a). The recovery of the old vegetation at Kodiak has been detailed by Griggs (1918). The first appearance of plants in secondary deposits of water-laid ash in Katmai Valley was described by Griggs (1919 b). The chemical conditions pre-sented by the ash to new plants in respect to its water-soluble salts, its toxic ferrous iron, its acidity, and its nitrogen content together with analyses of the rainfall to determine the amounts of ammonia and nitrites thus added to the soil have been reported by Shipley (1919 a, b, c).

The facts of most significance for our present problem brought out by these investigations may be summarized as follows:

1. The climate of the region as measured at Kodiak (Lat. N 58, Long.

W 153) more fully discussed by Griggs (1918, pp. 17–24), is equable, humid, and relatively mild except for the frequency of high winds. The monthly mean temperatures of the growing season, which is about 150 days in length, range from 40 to 60° F. The same month may, however, show a variation of nearly 10° from one year to another. But on account of the long days consequent upon the high latitude, the diurnal variation is small and the monthly means give a more adequate picture of the actual temperatures encountered by plants than in many regions. The extreme maximum temperature recorded is 82° F., and the extreme minimum − 12° F. The rainfall, most of which comes as fine mist, is well distributed and averages about 60 inches per annum. The skies are commonly overcast, and the hours of sunshine are relatively few, but there are no records of this important factor. Relative humidity is high, and evaporation low—about one-third as great as on Long Island, New York.

2. The explosion of Katmai, though of the utmost violence, largely because it was of the utmost violence, did not kill the underground parts of plants even on the slopes of the volcano.

3. Incandescent ejecta that poured into the Valley of Ten Thousand Smokes in the very much less violent eruption which produced that area, burned all vegetation therein to cinders.

4. Revegetation by the recovery of antecedent plants is entirely different from colonization by new plants. In studies of this sort great care must be taken to recognize hold-over plants and to distinguish them from new colonists.

5. It is only in areas permanently covered with a blanket of ash too thick to be penetrated by shoots from surviving roots that there is opportunity to study revegetation proper, i.e., the colonization of the ash by new plants.

6. The rainfall of the Katmai district is almost without ammonia. All determinations on uncontaminated rainfall except one gave only "traces" of ammonia. The one determination to which a value was assigned was 0.015 of one part per million, one-thirtieth of the fifteen-year average at the Rothamsted Experimental Station, which was given as 0.450 of one part per million. But rain which in falling had mixed with the vapors of the fumaroles from the Valley of Ten Thousand Smokes was strongly contaminated with ammonia. There is thus a *possibility* that ammonia from the fumaroles could be carried to the liverworts. The area where they grow is, however, across the mountain range in a different climatic province from the Valley of Ten Thousand Smokes and in the same region where the analyses summarized above were made.

7. Nitrous nitrogen was positively present in all rainfall determinations except one. The quantities, however, were minute, ranging from 0.004 of one part per million down. Nitrates were not determined owing to lack of a suitable field method at the time the work was done.

8. The ash as it fell was without nitrogen compounds and so remained for a long time. A Kjeldahl determination on a representative sample five years after the eruption gave the total nitrogen as 0.05 of one part per million or one part in 20 million. The "nitrogen free" sand used by Hellriegel in his classical experiments that demonstrated the capacity of legumes to fix atmospheric nitrogen where ordinary plants failed, contained 4 mg., of nitrogen per kg., 80 times as much as Shipley found in the ash of Katmai.

REVEGETATION OF OTHER VOLCANIC AREAS

These papers, especially Griggs (1918), contain a digest of the literature on the revegetation of volcanic areas. This need not be repeated here· Most of the accounts of revegetation of other volcanic areas have had to do with conditions comparable to those at Kodiak, one hundred miles from Katmai Volcano, where the ashfall was only ten inches, rather than with those found in the more deeply buried district nearer the crater. That is to say, they have described the recovery of antecedent vegetation rather than the colonization of bare ash.

The only pumice fields on which a serious attempt was made to study real revegetation as distinct from the recovery of old vegetation were at Krakatau. Much has been written about Krakatau, but as has been pointed out by Backer (1929; see also Hill 1930, Griggs 1918, p. 9, and Griggs 1930), the field work at Krakatau in the earlier years was insufficient to support any very detailed conclusions regarding the course of revegetation, or the factors concerned. For á detailed and critical review of the literature on Krakatau the reader should consult Backer's book.

Three pieces of information have, however, come out of the work on Krakatau which are of importance from our present point of view.

1. Blue-green algae were the pioneers, forming a gelatinous hygroscopic layer over much of the surface of the raw pumice. Treub 1888, collected and named six species of these plants. Later Raciborski (see Penzig, 1902) collected other species, apparently from similar habitats. These observations take on added importance in view of demonstrations by several workers (see Allison and Morris, 1930 and Jones, 1930) that certain members of the ·blue-green algae when alone or in symbiosis with bacteria are able to fix atmospheric nitrogen.

2. From samples of soil collected by Ernst, de Kruyff isolated a nitrogen-fixing bacterium. No extensive studies were, however, made to find out in detail the distribution of this organism over the area, or to what extent it was responsible for furnishing nitrogen to the new flora. At Krakatau, as at Katmai, leguminous plants with their bacterial nodules were of subordinate importance.

3. Liverworts played no part in the colonization of Krakatau. Campbell (1907) who visited the island in 1906, twenty years after the eruption, says on this point: "In a day's collecting, keeping a special lookout for

Hepaticae, the writer was unable to find a single specimen, and as we have already stated none have been reported by other collectors.   Inasmuch as Krakatau is within sight of Java and Sumatra, both of which have an extremely rich hepatic flora, the absence of these plants from the new flora of Krakatau is, to say the least, worthy of notice."   Since Raciborski had collected a specimen of *Anthoceros* in 1897 (see Penzig, 1902) Campbell is technically in error in stating that no liverworts had been found prior to 1906.   Nevertheless, Raciborski's single specimen of *Anthoceros* scarcely weakens Campbell's general conclusions.

Later collectors, working after the pioneer stages had passed, reported that numerous hepatics had then become established on Krakatau (see Backer, 1929).

PREVIOUS ALLUSIONS TO LIVERWORTS AS PIONEERS

It is quite certain that liverworts have not been hitherto reported as important colonists on volcanic ash.   But it is diffcult to ascertain to what extent they have been recognized as pioneers on normal terrains.   There are in the literature innumerable brief allusions to "mosses and liverworts as pioneers," but to date I have not found a detailed or extended study of the matter.   Brinkman (1929) indeed, making some observations on the habitat preferences of liverworts, using them as guides to ecological conditions on forest sites says: "So far as I know the work done this year though brief is new."

As reported at the time (Griggs, 1919 *b*), the beginnings of revegetation in Katmai Valley were confined to deposits of secondary ash carried into place by running water or built up like snowdrifts by the wind.   Where the ash lay undisturbed in the original falls it remained altogether bare. Considerable curiosity as to the cause of this fact was aroused at the time of the earlier studies, but its full significance could not be appreciated until it was emphasized by the progress of revegetation during the eleven year interval since the earlier observations.

In 1930 the secondary deposits were uniformly clothed with vegetation of one sort or another except where their surface was still too unstable to permit plants to mature.   On low waterlogged flats the dominant colonists were sedges, carices of several species which outside the devastated area found their place around the margins of shallow ponds.   On the well drained uplands the most characteristic colonist was blue-top grass, *Calamagrostis scabra*, which dominates the most favorable habitats throughout the district.

The vegetation of the ash drifts in 1930 was a marked contrast to that of 1919.   In the earlier year some of the hillsides were occupied by hundreds of lupines practically to the exclusion of other plants.   At that time it was expected that the nitrogen supply of the new soil would be built up from these leguminous plants, especially since their roots were richly supplied

TEXT Fig. 1. Drifted ash colonized by plants. Upper Katmai Valley. In contrast to the sterile pure ash beyond and to the left, the drift to the right contains a little soil as well as ash. To this is attributed the ability of the plants to catch hold. The gaunt skeletons of the alders, birches, and poplars killed by the eruption 18 years before still stand undecayed.

with large nodules. This expectation was not, however, realized. In 1930 lupines, though abundant in the old soil were very scarce on the ash, playing no part of consequence in colonizing even secondary ash deposits, while from pure undisturbed ash they were entirely absent.

It is foreign to the purpose of this paper, however, to discuss the revegetation of secondary deposits except as they throw light on the colonization of the pure ash. It was astonishing how slight a contamination with old soil would permit the establishment of plants. Text figure 1 is a photograph which brings out the situation. The bank at the right where the plants were growing was an ash drift deposited by the wind while the bare area to the left was stratified ash lying as it fell. The plants in the drift were thrifty though their youth showed that the drift had been colonized only recently. But not a single plant of any kind had obtained a foothold in the undisturbed ash to the left. Superficially, the ash of the two habitats bore much the same appearance. The difference which made possible the growth of plants was revealed when portions of the drift and of the undisturbed ash were shaken up with water in test tubes. The pure ash settled at once to the bottom leaving only a small amount of suspended matter in the nearly clear liquid above. But the drift contained considerable finer material which required several seconds to settle out. Again the matter remaining in suspension ten seconds after shaking was quite different in the two tubes. The water above the unmixed ash was a pearly gray, while that above the drift ash was a dirty brown. At the end of two hours the water above the drifted ash was still roughly twice as turbid as that in the other tube. Evidently the drifted ash contained a modicum of old soil lodged with the ash, and this was the constituent which made the growth of plants possible.

Here was an epitome, exaggerated almost to the point of caricature, of the whole problem of revegetation. Clearly enough here and elsewhere, it was the old soil and presumably the organic matter of the old soil which enabled plants to take hold. But in what way did the organic matter help the plants? Everyone recognises the value of organic matter in a soil. Volumes have been written on the subject. Yet we have no clear understanding of the matter.

Thinking that it might be possible to learn something from these two contrasted habitats of the function of organic matter in the soil, comparative studies have been made of the micro-flora, of the water-retaining capacity, and of the nitrogen content of these two "soils." N. R. Smith (results in process of publication) found no significant difference in the microörganisms.

To ascertain how far the water-retaining capacity of the ash drift had been modified by its contamination, I took weighed portions of ash from the drift and from the original ash-fall adjacent, baked them in a hot oven to remove all water, weighed again, wet them thoroughly with measured

TEXT FIG. 2.   A clump of grass (*Calamagrostis scabra*) established close to Novarupta.   Ammonia from the fumaroles in the middle distance is believed to be the factor which permits the establishment of higher plants here.   Nowhere in the Valley of Ten Thousand Smokes except in the vicinity of these particular fumaroles, around which ammonia salts were formerly deposited in quantity, is there a single seed plant.

amounts of distilled water, and allowed them to dry out gradually. For comparison, a sample of garden soil from Washington, one of pure white sand, and one of commercial peat were simultaneously tested. When the sand had entirely dried out, losing 100 percent of its water, and the pure ash retained only 4 percent the drifted ash still retained 10 percent as compared with 21 percent in soil and 42 percent in peat. In an arid climate, where the soil is in danger of drying out between rains, this difference might be critical to plant growth. But its importance in the Katmai district, where rain and fog are prevalent, is more doubtful.

The nitrogen content of the ash drift in question was kindly determined for me by Mr. Ready. Using the Winkler boric acid modification of the Kjeldahl method he found 109.2 parts per million of total nitrogen in the drift as compared with 15.6 parts in the unmixed ash adjacent. Both samples were negative to the diphenylamine test for nitrates, but both gave a small quantity of ammonia,—the wind-blown ash being recorded as 0.6 parts per million and the undisturbed ash as 0.5 ppm. One must have considerable hesitancy in evaluating these results. If all the nitrogen were in a form available to plants the difference in the habitats would be explained. But we do not know the extent to which the complex organic nitrogeneous compounds of such a soil are available.

Another situation, however, found over the range, in the Valley of Ten Thousand Smokes, lends support to the belief that it is the nitrogen rather than microörganisms or soil colloids which is the critical factor.

Close to Novarupta volcano, in the very heart of the most completely devastated portion of the whole district—in an area which was buried to a great and unknown depth under incandescent ejecta—a number of isolated clumps of grass, *Calamagrostis scabra*, have come up (see text fig. 2). These grasses are the more significant because, except for them and for mosses, liverworts, and algae around some of the fumaroles, there are no plant colonists whatever in the Valley of Ten Thousand Smokes, nor on the mountains which surround it. Outside this special area there is not a blade of grass for miles around.

Very clearly, the grass clumps around Novarupta must owe their existence to some favoring factor here present but absent elsewhere. This factor is, I suspect, the presence of ammonia in the emanations of the fumaroles of this particular area, for it was here that Shipley discovered in 1917 masses of ammonium chlorid crystals sublimed by the fumes. It should be remembered, however, that in the absence of ammonia determinations in 1930, this supposition is only a conjecture. But if this is correct, the occurrence of grasses around Novarupta and in soil-contaminated-ash drifts would emphasize the belief that it is the lack of nitrogen compounds in the pure ash which has prevented the growth of flowering plants there.

TEXT FIG. 3. Rosettes of moss *Pogonatum* and seedlings of willow come up on an old liverwort turf. *Dicranella* is also present but cannot be made out in the photograph. The willows though small had survived two or three winters and would probably become established in time. The liverworts show no tendency to grow over the fragments of twigs and bark on the ash surface as the mosses do.

LIVERWORTS, THE PIONEERS ON PURE ASH

On the original undisturbed ash the pioneer vegetation consisted entirely of leafy liverworts of the family Jungermanniaceae. These small and seemingly delicate forms have occupied considerable areas where no other plants have been able to obtain a foothold. Their growth has become thick and heavy, the shoots densely felted together into continuous unbroken carpets half an inch thick. Where a little depression conserves moisture somewhat, the liverworts are bright green. But on the general surface, which is subjected to periodic desiccation, they take on a dark brown or nearly black color. In the field, therefore, the liverwort carpet was termed the "black layer" (see text fig. 3).

On account of the small size of the plants comprising the black layer and the density of their growth, its true character was not apparent except upon examination with a lens. Its general appearance was not unlike the growth of moss protonema commonly found on clay banks, but it was far thicker and more massive than protonemal growths ever become.
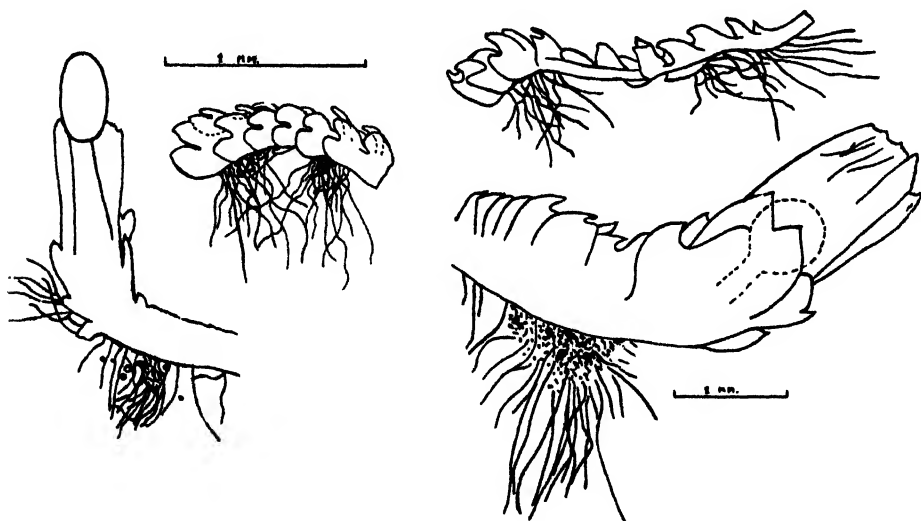
TEXT FIG. 4. Vegetative and fruiting branches of liverworts teased out from the black layer. Left, *Cephaloziella byssacea* (Roth) Warnst, twenty-five times natural size; right *Lophozia bicrenata* (Schmidt)Dumort, fifteen times natural size.

There were two species of liverworts. One was much more delicate than the other, and so they were called in the field the "big liverwort" and the "small liverwort." Specimens kindly determined by Dr. Alexander W. Evans proved to be, respectively, *Lophozia bicrenata* (Schmidt) Dumort and *Cephaloziella byssacea* (Roth) Warmst—(*C. starkei* (Funk) Schiffn.). Of the two, the latter seems to be the more important. It is one of the smallest and most delicate of liverworts, not revealing its characteristics except under considerable magnification (text fig. 4).

TEXT FIG. 5.   General view of the liverwort turf.   Where erosion, cutting through the ash layers, lays bare the old soil as at the left, grass and other plants grow luxuriantly.   Although many seeds of such plants must lodge on the ash, nothing but the liverworts has gained a foothold there.

Both *Lophozia* and *Cephaloziella* were found fruiting copiously in patches several square yards in extent.   The ability of the liverworts thus to complete their life cycle in normal fashion on the ash takes in added significance from the fact, discussed below, that the hair cap mosses, *Pogonatum*, which follow the liverworts are unable to mature spores in the same habitat (text fig. 4).

Where areas covered with the black liverwort layer were cut by erosion into gullies in which the old soil beneath the ash was exposed, a most interesting condition occurred.   The old soil supported a thrifty growth of higher plants of many species, grasses, lupines, equisetums, fireweed, artemisias, and others.   The reproductive bodies of these plants, which were fruiting normally, must have lodged in quantities in the liverwort carpet adjacent, but none of them had gained a foothold there (text fig. 5).

The liverwort layer

Where the liverwort layer was thickest and heaviest, it had crinkled up irregularly and broken away from the ground here and there.   This buckling up seemed to be due to continued growth after the whole surface was occupied.   Like liverworts generally, these were provided with rhizoids which thoroughly permeated the top layer of ash and clasped the grains to the depth of nearly an inch, so that when pulled off the ground the liverworts came away as a sod.   It need hardly be said that such a growth of liverworts has never been seen by the writer elsewhere.

One of the first questions concerning the liverwort layer was its age. We knew that the areas occupied had been bare eleven years earlier, but we did not find means of determining what fraction of this interval had been occupied by the development of the black layer.   Certainly, however, it was no ephemeral growth.   It had come through the winter in practically the same condition as when found.   Likewise, it made no noticeable extension during the period it was under observation.   Very possibly its development had required the greater part of a decade.

At the time of our observations, the liverwort layer had extended about as far as the stability of the ground permitted.   It had evidently started in the shelter of windbreaks, such as fallen logs and from there had spread out over the ash nearly as far as the wind left the surface stable enough for its growth.   At the edge of its growth the black layer was, in some places, extending farther onto the bare ash.   At others it had been broken up or killed back by the elements.   Evidence of the effectiveness of the liverwort layer as a soil binder was abundant.   In many places where it had been broken through, the wind had scooped out the loose ash in the unprotected hole beneath, to a depth of two or three inches.

With the gradual spread of the liverwort layer on surfaces inhospitable to higher plants, and the increase of grass and forbs on the secondary deposits, the areas of loose ash become less every year.   And reduction in

9

bare ash area means reduction in the quantity of drifting ash. So the country is becoming less and less unstable as greater and greater areas become possible habitats for plants of one sort or another. Beginning imperceptibly in the earlier years, the stabilizing forces have now gained such momentum that rapid progress in revegetation may be expected in the years to come.

Physical and chemical conditions of the liverwort habitat

The liverwort beds were studied most extensively on the flat at the mouth of Martin Creek, about ten miles south of the volcano. There the ash ranged from 2 feet to 20 inches in depth. Most of the fall consisted of rather coarse fragments of pumice mixed with rock crystals freed from the lava by the violence of the explosion. There were three layers corresponding to the three main phases of the eruption. These differ somewhat in color and character, but are sufficiently similar in chemical composition to be treated together as analyzed by Elton Fulmer from a sample of the fall at Kodiak:

Loss on ignition, 0.65%; silica, SiO_2, 72.16%; ferric oxid, Fe_2O_3, 2.85%; manganese oxid, MnO-O, 41%; titanium oxid, trace; alumina, Al_2O_3, 13.85%; lime, CaO, 3.80%; magnesia, MgO, 0.47%; soda, Na_2O, 3.86%; potash K_2O, 2.43%; sulfuric acid, SO_3, 0.20%; phosphoric acid, P_2O_5, 0.36%.

In addition to the elements shown by ordinary analyses such as that given above, more refined methods developed by Zies, 1929, have revealed the presence of many other elements including cholorine, sulfur, barium, fluorine, copper, lead, tin, molybdenum, arsenic, antimony, cadmium, thallium, zinc, and gallium. Inasmuch, as Zies was studying only the metallic constituents of the lava, and did not look for other elements, his work makes it seem probable that most of the other elements known to chemists are also present. It is thus likely that the ash contains sufficient amounts of all those elements, such as boron and iodine, minute quantities of which have been shown by recent researches to be necessary to plants.

The top of the deposit is fine dusty sand, the last material to settle out of the air after the eruption. The lower coarser strata present very much the same physical conditions for drainage and for root penetration as would be afforded by a pile of loose cinders.

Bare ash and ash immediately below the liverwort beds gave a neutral reaction, pH 7. Among the plants or their rhizoids the reaction was acid, pH 5–6.

Nitrogen determinations made by Mr. Ready on specimens brought back in cloth sacks gave: for clean ash below the liverwort layer, ammonia 0.6 ppm., nitrates none, total nitrogen 15.0 ppm.; for fine grained ash adjacent to the liverwort bed but bare, ammonia 0.9 ppm., nitrates none, total nitrogen, 42.6 ppm. While the amount of nitrogen compounds revealed by these analyses is substantially higher than that found by

Shipley thirteen years earlier, it is still very low.   The total nitrogen in the soil of the Department of Agriculture experimental farm at Arlington, Va., which is counted a rather poor soil is 8,000 to 10,000 ppm., 200 to 250 times greater than the highest of these analyses.

Determinations of the nitrogen of the liverworts themselves are rather variable and unsatisfactory because the rhizoids cling so tenaciously to the particles of ash that it was impossible to secure a clean sample consisting of liverwort alone without foreign ingredients.   Three determinations, however, gave: 8,000, 9,300, and 15,300 parts per million of total nitrogen, all of them probably under rather than over the true figure, for the reason stated.   These quantities indicate a nitrogen content in the liverworts comparable with that of wheat straw.

If all of the nitrogen of the ash were in a form available to the liverworts, and if the supply were being replenished from time to time from some outside source it is thus not impossible that the liverworts, if they grew very slowly, might secure the nitrogen necessary for their growth from the ash.

Algae as indicators of available nitrogen

The best and one of the most convenient tests for available nitrogen are plants themselves.   Through the kindness of Dr. F. E. Allison of the Fixed Nitrogen Laboratory, I obtained a pure culture of one of the green algae, a species of *Chlorella*, used by F. B. Wann on his studies of nitrogen fixation. This alga and the related form, *Chlorococcum*, normally found with the liverwort (see below), are exceedingly good indicators of available nitrogen. They cannot grow in nitrogen-free media, but will thrive in cultures with very small amounts of nitrogen compounds, down as low as two parts of nitrogen per million.   This well known fact has been confirmed repeatedly in cultural work on the liverworts in my laboratory.   I have accordingly come to make considerable use of these algae as checks on the composition of media.

An example will illustrate the point.   Three portions of each of the ash samples analyzed by Mr. Ready were placed in saucer-shaped culture dishes and inoculated with algae.   One of the dishes was moistened with Shive's three salt nutrient medium, as found best for wheat "R_5S_2"; the second was moistened with a similar solution in which calcium sulfate had been substituted for calcium nitrate, being thus nitrogen-free; and the third with distilled water.   In the course of a month the algae covered the surface of the dish provided with nitrate, while the other dishes remained clear and free of algae.   It should be noted also that in mixed cultures including liverworts, mosses and algae, the liverworts grew well in the nitrogen-free dishes, but in the nitrate cultures they were overgrown and crowded out by the mosses and algae.

Contaminations of the liverwort layer

The liverwort layer may be described as a pure stand of liverworts, in the sense that such a term is used by the foresters, *i.e.*, no other plants of comparable size occur with the liverworts. But it is not a pure culture in the bacteriologist's sense. In it must lodge not only seeds of most of the plants of the vicinity, but spores of all the lower organisms of the region, fungi, mosses, algae, and bacteria. Microscopic examination revealed small growths of fungus hyphae, moss protonema, and green algal cells. These were *Chlorella*-like forms which produced zoöspores freely in culture and answered to the description of *Chlorococcum humicolum* (Naeg) Raben. given by West and Fritsch in their British Fresh Water Algae.

One expected constituent of such a complex, however, was absent. There were no blue-green algae, Cyanophyceae. Diligent search through all the specimens examined failed to reveal a single individual of this group. This field diagnosis has since been confirmed by the cultures in the laboratory. All of the organisms observed among the liverworts in the field, with the addition of bacteria, which were not noted under the magnifications used in the field, have appeared as contaminations in the cultures. But there were no blue-green contaminations for three months—not till after the classes in general botany studied Cyanophyceae, in an adjoining laboratory, when a few contaminations appeared in saucer cultures frequently opened to the air, and then on the bare surface of the culture medium, rather than among the liverworts planted in the middle of the saucer.   .

The absence of the blue-green algae in the black layer was, to me, most surprising. Almost any mossy ground in lower latitudes would contain considerable numbers of them. The whole Katmai district, however, seems to be poor in Cyanophyceae. These algae, as is well known, are generally the most characteristic vegetation of hot springs, but there are many hot springs around the Valley of Ten Thousand Smokes which are without algae, and harbor no plants of any kind other than iron bacteria, which form a copious ochraceous deposit around them. Others have blue-green algae in addition, but they do not appear in any large quantity or variety.

Possibility of symbiotic relations

In the field the other organisms found with the liverworts, mosses, algae, and fungi were suppressed and remained of microscopic proportions under the conditions where the liverworts grew to best advantage.

One exception to this statement, however, needs to be noted. In several places areas of a square yard or more were found thickly covered with the yellow ascocorps of a fungus, which seemed to be drawing its nourishment, directly or indirectly, from the liverwort layer.

The presence of fungi among the liverworts raises the mycorrhiza question. It is quite possible that the capacity of the liverworts for living

on a nitrogen-poor medium depends on a symbiotic relationship with some nitrogen fixing fungus, or bacterium.   Facilities for reaching a decision on this question were not at hand in the field.   But it is being carefully considered in the cultural studies now under way at Washington.

Liverworts unable to draw nourishment from a distance

One characteristic of the liverworts which is of importance in studies of their nutrition should be pointed out.   Because of the absence of roots or any elongated absorbing organs the liverworts must draw their nourishment from their immediate surroundings.   The rhizoids do not extend more than an inch into the ground.   So, after allowing for capillarity, there is no possibility of the liverworts obtaining food from scattered organic debris which might be reached unobserved by an extensive root system.

Liverworts on other substrata than the ash

The black liverwort layer was not confined to the pure ash.   On the contrary, once its importance had been recognized, it was found that similar growths pioneer the ground in many other habitats.   Many acres of waterlaid ashflats near the mouths of Katmai River and Martin Creek were carpeted with a similar liverwort felt except that here, with the liverworts were other plants: mosses, equisetums grasses, forbs, and shrubs.   In such situations other liverworts than the two characteristic of the pure ash also came in.   In specimens from such a locality Dr. Evans reports *Scapania undulata* (L) Dumort and *Anthelia julacea* (L) Dumort while *Cephaloziella byssacea* was replaced by *Cephalozia bicuspidata* (L) and *Lophozia bicrenata* by *L. alpestris* (Schleich) Evans.

The debris of Great Mageik Landslide (see Griggs, 1920) consists of rock fragments in part comminuted practically to the consistency of soil.   But though it was contemporaneous with the eruption and is now 18 years old, it has not been occupied by flowering plants except around the terminus where the debris contains soil as well as rock fragments.   In the upper portion, the only vegetation consists of liverworts which, here as in the ash, have formed continuous carpets over considerable areas.   Here as in waterlaid-ash other liverworts than the two of the pure ash have come in. Dr. Evans reports from this area: *Cephalozia biscuspidata* (L) Dumort, *Lophozia bicrenata* (Schmidt) Dumort, *Scapania mucronata* H. Buch and *Nardia scaleris* (Schrod) S. F. Gray.

A number of mildly steaming areas in the Valley of Ten Thousand Smokes were carpeted with mats of liverworts punctured by holes an inch or two in diameter through which a little steam exhaled.   A number of tests with the thermometer showed the liverworts growing at temperatures up to 23° C. (73° F.) which is warm for the region.   But no higher temperature could be found among them.

At Kodiak also there are indications that deep secondary deposits which were not immediately penetrated by shoots from hold-over roots, were

likewise colonized by liverworts. At "station 49," which is a high, well drained situation offering considerable obstacles to revegetation, the ground was covered in 1930 with a black liverwort layer similar to that on the mainland. Again at "station 14," a wind-blown ash drift which remained bare for the first seven years, there were remnants of a liverwort layer in bare spots in the grass which had nearly covered the ground. The liverworts here were determined by Dr. Evans as *Nardia scalaris* (Schrod) S. F. Gray and *Jungermannia Sphaerocorpa* Hook while a *Cephalozia* or *Cephaloziella* also came up in a culture from this material. Furthermore on going over my field notes of 1919 I find records at these and other places of a "scanty growth of brown protonema." As this was not examined microscopically it was very probably the beginning of the liverwort layer.

Again on cutbanks where the subsoil was exposed and in the crevices of the rocks on mountain crests tufts of liverworts similar to those coming in on the ash were found.

Bare spots on the tundra outside the devastated area are likewise often covered with sheets of matted black liverworts in all respects similar to the liverwort layer on the ash. It appears, then, that the liverworts are not special to the ash but, under the climatic conditions obtaining, are in fact pioneers of new ground generally.

Relations of the liverworts to succeeding plants

Where conditions are even a little more favorable than on the most exposed bare ash deposits the moss protonema, always present in small quantities with the liverwort, begins to grow and produce the mature plants. The first moss to come in is a *Dicranella*. This frequently matures sporophytes and these are normal in appearance. A little later *Dicranella* is followed by a *Pogonatum*, which because of its greater size is the more conspicuous. *Pogonatum* does not, however, thrive on the ash. On pure ash in deep deposits it does not fruit. On the thinner ash layer at Kodiak and on contaminated water-laid ash fruiting occurs only seldom and then the capsules are usually aborted and come to nothing. The stunted growth and scanty aborted fruits of the moss contrast sharply with the luxuriant growth and copious well developed fruits of the liverworts. Very evidently the mosses starve in conditions that are favorable to the liverworts. In their relations to fallen twigs and similar debris the mosses are likewise very different from the liverworts. While the liverworts take no advantage of such materials and even seem to avoid them the mosses very definitely follow along them as though gaining some nutriment from their decay.

Some of the willow seedlings which start everywhere soon after the seeds are mature were able to persist in conditions a little less extreme than those of the pioneers. Stunted little willow plants a few inches tall were found in such places which had succeeded in holding on for four or five years (text fig. 3).

The story of the liverworts' place in succession is apparently completed by conditions found near the mouth of Katmai River where the ground is more sheltered and the ash was earlier occupied by the liverworts. Here *Nardia scalaris* (Schrod) S. F. Gray had come in in addition to *Cephaloziella*. The mosses had grown strong and rank, *Pogonatum* being replaced by *Polytrichum*. The willow seedlings had grown two or three feet tall and other plants such as *Calamagrostis scabra* and *Artemisia tilesii* had come in. Very evidently this area will soon develop into a willow thicket and so enter the normal succession characteristic of the region.

The liverworts, then, seem to make possible the advent of other plants. The organic matter produced by their growth over a period of years probably gradually accumulates until it becomes sufficient for ordinary plants and so a true soil may be said to be initiated.

Yet conditions so far observed do not warrant too confident assertion of the ability of the liverworts alone to build up the organic basis of a soil. The succession of seed plants after the liverworts was observed only in an area sheltered from the full fury of the eruption where the trees had not been killed. In addition to the liverworts the leaves of the trees have accumulated on the ash during the interval since the eruption. Thus in this place the liverworts have not been the only source of organic matter for the new soil.

In order to make sure that the liverworts alone can build up the humus required for soil formation it will be necessary to observe in later years the sequence of events in exposed situations now occupied by liverworts alone.

CONCLUSIONS

We may conclude, I think, that these liverworts, especially *Cephaloziella byssacea*, are plants of very low nitrogen requirements. Their dominant position as pioneers on the nitrogen-free ash appears to be due to their ability to thrive on a lower concentration of nitrogen compounds than the other plants, algae, mosses and seed plants whose dissemules reach the habitat.

Further than this field studies alone will not permit us to go. To determine just how low their nitrogen requirements may be, whether they are able to utilize free atmospheric nitrogen, or whether their nitrogen metabolism is assisted by some nitrogen-fixing symbiote are questions which can be answered only by cultural work under controlled conditions. Such work is now under way, but has not yet progressed far enough to admit of definite conclusions. It may be stated at this stage, however, that nothing has yet developed in the cultures to modify the conclusions drawn from field study. *Cephaloziella* can certainly grow and increase for a time at least on media as nearly free from nitrogen compounds as it is possible to prepare.

SUMMARY

The ash erupted by Katmai in 1912 furnishes an exceptional opportunity to study on a large scale the colonization of a "soil" free from organic matter and from organisms. Even a very little organic contamination, as in wind-laid ash drifts, will permit the growth of plants. But ordinary plants cannot colonize undisturbed deposits of pure ash. The critical factor appears to be lack of nitrogen. The ash is practically nitrogen-free.

At the time of former visits in 1915–1919, uncontaminated ash deposits were bare of vegetation, but on re-visiting the area in 1930, again under the auspicies of the National Geographic Society, it was found that extensive areas were covered with a thick matted carpet of leafy liverworts in a pure stand. There were two species, of which *Cephaloziella byssacea* appears to be more important. Both species fruited copiously on pure ash. They were evidently able in some way to secure the nitrogen necessary for the construction of their protoplasm. Cultural studies to determine, if possible, the means by which the liverworts secure their nitrogen are under way.

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A LYCOPODIACEOUS STROBILUS FROM THE POCONO SANDSTONE OF PENNSYLVANIA

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Although several instances are known of the occurrence of lycopodiaceous fossils in the pre-Coal Measures rocks of North America evidences of their fructifications are exceedingly rare. For this reason a specimen recently found in the Pocono sandstone and which shows some of its internal organization is of considerable interest.

This specimen, along with several other plant fossils, was discovered during the summer of 1931 in a small quarry about one and one half miles northeast of Port Allegany, McKean County, Pennsylvania. After they were collected these fossils had come into the possession of Mr. J. C. Galloway of Port Allegany, who, appreciating their possible scientific value, very kindly submitted them to the present author for study. Unfortunately the strobilus had undergone no petrifaction, with the consequence that many of the outstanding features of its internal organization were lost or hopelessly obscured. On the other hand certain structural features are preserved in a remarkable fashion.

The strobilus, as shown in text figure 1, measures one and three-eighths by four inches. The sides are nearly parallel and the apex is bluntly rounded. Attached to it is a stout peduncle, about three inches long but incomplete, which appears to have had a pith. Whether or not this peduncle bore bracts cannot be determined, but the straightness of it suggests that the strobilus was originally borne in an upright position.

The upper half of the strobilus had undergone very little decay and the partial obliteration of the structure was caused rather by the severe flattening to which the strobilus had been subjected. A lengthwise split through this upper portion had exposed the axis (as shown in text fig. 1) and by carefully removing some of the attached sporophylls with a small chisel the sporangia could be removed. These sporangia are radially elongated and still retain their spores, but whether they are attached to the upper surfaces of the sporophylls by their full lengths as is characteristic of some lycopods or whether they are attached distally to specialized sporangiophores as in the Calamariae or in *Cheirostrobus* or distally to the sporophyll as in *Spencerites*, could not be determined. It is assumed, however, that the former situation existed since there is considerable evidence that the strobilus is lycopodiaceous and belongs to the Sigillariae.

The sporophylls are a little more than half an inch long and are borne at right angles to the axis. They are in whorls about three-sixteenths of an inch

apart and those in adjacent whorls alternate with each other. The exact number of sporophylls per whorl is indeterminable because of the distortion brought about by flattening, but they appear to have been numerous, probably twelve to twenty. This whorled arrangement of the sporophylls could be seen especially well where portions of the outer surface had been flattened against the enclosing sandstone. Also, at certain places along the exposed axis, the alternation could be seen.
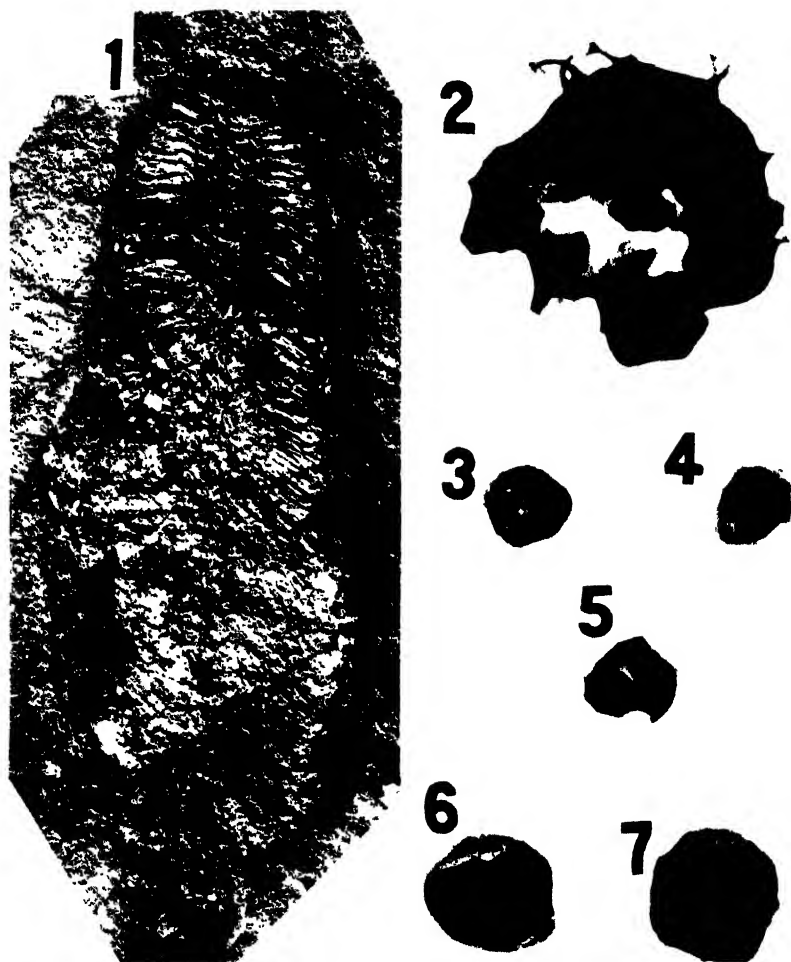
TEXT FIGS. 1–7. FIG. 1. Photograph of strobilus, natural size. The axis is exposed in the upper part. FIG. 2. Portion of megaspore showing appendages. Diameter 152 microns. FIGS. 3, 4, 5. Microspores of the smaller type. Figure 5 shows the triradiate markings. Diameter about 38 microns. FIGS. 6, 7. Larger microspores. Diameter about 76 microns.

The presence of spores of different sizes in the sporangia indicates heterospory. The microspores vary considerably in size. The smaller and

more numerous are around thirty-five microns (text figs. 3–5) but some of them are more than twice as large (text figs. 6–7). Nothing remains of any of the spores except the heavily cutinized coats. The triradiate markings showing where the four cells were originally joined together in the tetrad stage are visible on some (text fig. 5). The microspore walls appear to have been slightly rough but there is no evidence of their having had long appendages.

The megaspores are much larger and less numerous. None of them were found complete and the process of pulverizing the carbonized sporangia for mounting was exceedingly destructive to the appendages. Judging from what could be seen of the megaspores their diameter is two or three times that of the largest microspores, or approximately one hundred and fifty microns or more (text fig. 2).

The shape and position of the sporangia and the sporophylls indicate lycopodiaceous affinities rather than equisetaceous affinities for this fossil. Whether or not it is a true *Sigillaria* is at present undecided. According to Zeiller (4) the verticillate arrangement of the sporophylls in *Sigillariostrobus* is characteristic for the genus and serves as a means of distinguishing it from *Lepidostrobus*. He does attribute this feature to one species of *Lepidostrobus* of which, however, he expresses some doubts concerning its generic affinity.

Considerable interest centers about the exact geological age of the Pocono fossils. The Pocono sandstone is generally assumed to be the basal member of the Mississippian in northern Pennsylvania; but quite recently Chadwick (1), after making detailed studies of the lower Mississippian and Upper Devonian in that region and in southern New York, believes that the Pocono properly belongs to the Upper Devonian, thus placing the Devonio-Carboniferous boundary above that formation rather than below it as has been done heretofore.

The possibility of the Pocono belonging to the Upper Devonian is supported to a certain extent by the occurrence along with the lycopod strobilus of a species of *Archaeopteris* closely resembling specimens of *A. Roemeriana* Goepp. from the Upper Devonian of Bear Island. *Archaeopteris* occurs only in Upper Devonian rocks, as far as it is known, and is sometimes used as an index fossil in assigning formations to that age.

Three new sigillarian genera, *Helenia*, *Heleniella*, and *Amadokia*, recently described by Zalesskij (3) from the Upper Devonian rocks of the Donetz Basin of south Russia, are of some interest in connection with the Pocono material. To *Helenia*, Zalesskij would assign the well-known specimen of *Archaeosigillaria primaeva* (Rogers) White from the Portage formation of New York (2). He recognizes a similarity between this and some of his newly discovered Russian material which, however, differs from the material upon which the original description of *Archaeosigillaria* was based. This possible similarity of the Russian and American material is of some signifi-

cance in indicating a similarity of the flora between these two widely separated areas during Upper Devonian times.   Also the Russian material lends strength to the sigillarian interpretation of the Pocono strobilus as well as to the probable Devonian age of the Pocono by indicating that the remains of the Sigillariae can be expected to occur in Devonian rocks.

SUMMARY

The specimen here described is an unpetrified lycopodiaceous strobilus from the Pocono sandstone near Port Allegany, McKean County, Pennsylvania, which is possibly of Upper Devonian age.   The whorled arrangement of the sporophylls suggests sigillarian affinities.   Both megaspores and microspores are present; the former measure approximately 150 microns while the latter are smaller but quite variable in size.   The recent discovery of sigillarian remains in the Upper Devonian of the Donetz Basin is a coincidence of some probable significance in connection with this.

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BRACTEOLA, A NEW GRASS GENUS FROM AFRICA

Jason R. Swallen

Among some unnamed African grasses in the U. S. National Herbarium collected by Dr. John Gossweiler in Angola, Portuguese West Africa, was one distinct from any known genus. The name given to it is the Latin word meaning "a small leaf of gold," referring to the light golden, shining, compressed spikelets.

Bracteola Swallen, gen. nov.

Spiculae 2-florae in rhachi spicarum unilateralium sessiles, 2-seriatae, rachilla supra glumas articulata ultra flores producta, flore inferiore hermaphrodito, superiore masculo vel sterili; glumae aequales quam flores longiores, carinatae, uninerves, prima angusta non aristata, secunda latior sub apice brevissime aristata; lemma inferius firmum, carinatum, 3-nerve nervis villosis, sub apice aristatum, lemma superius reductum, glabrum. Stamina 3. Gramen perenne, glabrum, basi decumbens nodis radicans, culmis et vaginis compressis, laminis conduplicatis. Spicae 4, digitatae suberectae.

Spikelets 2-flowered, the lower floret perfect, the upper staminate or neuter, sessile in two rows on one side of a three-angled rachis, the rachilla disarticulating above the glumes, prolonged beyond the upper floret as a slender bristle; glumes equal, acute, keeled, 1-nerved, the first narrow, bowed out below, awnless, the second much wider than the first, short-awned from below the apex; lemma obovate, firm compressed-keeled, 3-nerved, the nerves densely villous, the lateral ones marginal, awned from the back just below the apex; palea acuminate, two-keeled, a little shorter than the lemma; upper floret reduced, the lemma thinner, glabrous, frequently bearing a staminate flower.

Slender, glabrous perennial with compressed culms and sheaths, short conduplicate blades, the upper ones much reduced, and long-exserted inflorescence of four digitate, suberect, one-sided spikes.

Type species, *B. lucida*.

Bracteola lucida Swallen, sp. nov.

Culmi graciles, adscendentes, compressi, glabri, 75 cm. alti; vaginae carinatae, glabrae, in ore pubescentes, quam internodia breviores; ligula 1 mm. longa; laminae conduplicatae, subtruncatae, 3–11 cm. longae, 3–8 mm. latae, laeves, glabrae, marginibus firmis scaberulis; spicae 4, digitatae, 8 cm. longae; spiculae 4.5 mm. longae, compressae; glumae subaequales, acutae, 1-nerves, in carina scabrae, prima angusta non aristata, secunda quam prima duplo latior, aristata, arista 1 mm. longa; lemma inferius 3.8 mm. longum, acutum, firmum, striatum, 3-nerve nervis villosis sub apice aristatum, arista 1.5 mm. longa; palea acuminata, 2-carinata, marginibus

hyalinis, quam lemma paulo brevior; flos superior masculus vel sterilis, glaber, 3 mm. longus, aristatus, arista 0.5 mm. longa.

Culms slender, ascending, sometimes decumbent at the base and rooting at the lower nodes, compressed, glabrous, sparingly branched above, 75 cm. tall; sheaths compressed-keeled, smooth, glabrous, except for a small tuft of hairs at the mouth, much shorter than the internodes; ligule 1 mm. long, membranaceous, shortly ciliate; blades conduplicate, subtruncate, sometimes apiculate, 3–11 cm. long, 3–8 mm. wide, scabrous on the firm, whitish midnerve and margins near the tip, sparingly pubescent near the

Text Fig. 1. *Bracteola lucida.* Inflorescence natural size; spikelet and floret × 10.

base, otherwise smooth and glabrous, the uppermost nearly obsolete; inflorescence long-exserted, the peduncles very slender, somewhat flexuous; spikes 4, digitate, suberect to spreading, 8 cm. long, the rachis narrow, three-angled, scabrous on the margins, terminating in a much reduced imperfect floret; spikelets 4.5 mm. long, compressed, pale golden, shining; glumes equal or nearly so, acute, 1-nerved, scabrous on the keel, the first bowed out below, 0.5 mm. wide from keel to margin, awnless, the second not bowed out below, 1 mm. wide from keel to margin, awned from below the tip, the awn less than 1 mm. long; fertile lemma acute, 3.8 mm. long, firm, minutely striate, 3-nerved, the lateral ones marginal, densely villous with ascending hairs as

much as 2 mm. long, awned from the back just below the apex, the awn slender, 1.5 mm. long; palea membranaceous with hyaline margins, acuminate, 2-keeled, a little shorter than the lemma; upper lemma slender, glabrous, awned, the awn about 0.5 mm. long, empty or sometimes bearing a staminate flower; prolongation of the rachilla 1 mm. long.

Type in the U. S. National Herbarium, no. 1526559, collected in the lowlands of Cassange, near the Lui River at Dunda, Angola, alt. 800 M., January 25, 1931, by Dr. John Gossweiler (no. 9613).

Bracteola is most nearly related to *Brachyachne* Stapf, differing in the lyrate, 2-flowered, awned spikelets with thinner glumes and firmer lemmas.

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A STUDY OF THE CARPOPHORE OF THE UMBELLIFERAE

Gemma Jackson

INTRODUCTION

The carpophore of the Umbelliferae is commonly referred to as an axis or as an axial structure (Lindley, Drude, Gray, Le Maout and Decaisne, Britton and Brown, Hutchinson, etc.). Certain authors go so far as to state definitely that the carpophore is a prolongation of the receptacle between the carpels. Most of them, however, merely call it "axial" or a "central axis." The axial theory of the nature of the carpophore is based apparently on its axis-like appearance and form. This theory, although commonly held, has not been universally accepted, and there have been brought forward other explanations of the morphology of the carpophore.

A second interpretation denies the existence of the carpophore as a separate structure and considers it composed of portions of the carpels. The carpophore is, therefore, appendicular in nature, according to the proponents of this second theory, among whom are von Mohl, Van Tieghem, Eichler, and Henslow. This theory rests largely on anatomical evidence—the position and course of the vascular bundles.

Other explanations of the carpophore have been proposed but none of these has been of sufficient plausibility to become commonly held.

Recent investigations in floral anatomy have clearly demonstrated the fallibility inherent in interpretations of floral structures based mainly on general morphological features. Careful studies of the vascular system of the flower have, in many cases, disclosed the true nature of floral parts which had previously been misinterpreted. The validity of such anatomical evidence is recognized today. It would seem, therefore, that a thorough investigation of the vascular structure of flowers and fruits of representative genera of the Umbelliferae should furnish evidence sufficient to establish the true morphology of the carpophore; and with this in mind the present study was undertaken.

Genera from the three subfamilies into which the family is divided were studied, these being so chosen as to include representatives of as many of the tribes as material available permitted. One or more species of twenty-five genera were studied.

For the examination of the material there were prepared complete cross-sectional series of flowers and fruits. In the case of most of the species studied, fruits of several ages were cut, including, when possible, mature fruits. Certain of the Umbelliferae show pleomorphism of the flowers with

11

respect to the male and female structures.   In the present paper only flowers with fertile ovaries are considered.

<div align="center">CARPOPHORE ANATOMY</div>

The carpophore is essentially a feature of the Apioideae.   In the Hydrocotyloideae and the Saniculoideae the carpophore is greatly reduced or absent, and when present does not become free at the maturing of the fruit. The descriptions of material examined and the discussion are, therefore, limited chiefly to the Apioideae.   The two other subfamilies are considered more briefly, and largely with reference to points which seem definitely related to the present problem.

Within the Apioideae, there was found considerable variation in the details of formation and in the histological nature of the carpophore.   This variation is not, however, of such a nature as to affect the fundamental morphology of that structure.   It seems best to discuss the carpophore in considerable detail in one species of this subfamily, and to describe less fully one or more species from other tribes (as far as available material and space allow).   The species chosen for most complete description was selected, mainly, because it has a large and obvious carpophore which is very "axis-like" in general appearance.   It should not be concluded that all the members of the subfamily are similar to those few which are described.   All features observed which are of importance in the present study are mentioned.   Many other differences are omitted.

<div align="center">Subfamily Apioideae.   Tribe Scandicineae
Example, Osmorhiza longistylis</div>

Flower Stage.   The pedicel shows a rather small amount of vascular tissue arranged in several bundles (fig. 3).   This vascular tissue increases in amount as the pedicel widens out into the receptacle, and forms a dissected hollow cylinder.   From this cylinder there pass outward into the cortex three bundles on each side (bundles nos. 1–2, 5–7 and 10 of fig. 4).   There are left two arcs of vascular tissue which constitute the rest of the stele. A short distance above this level these two arcs break up in the following manner: Four bundles pass outward from the original position of the arcs, two coming from each arc (bundles nos. 3–4 and 8–9 of fig. 5).   As these four bundles pass outward four small protoxylem strands, two from each arc, move inward nearer to the center of the flower (protoxylem strands nos. 11a, 11b, 12a, and 12b of fig. 5).   These four protoxylem strands are the last xylem strands of the vascular arcs and are accompanied, as they move inward, by the remaining parenchymatous tissue of the arcs (fig. 5).

The phloem, both in the pedicel and in the flower proper, is not sufficiently characteristic histologically at this stage to be distinguished with certainty. Each of the ten peripheral bundles is composed of a group of lignified xylem cells and an area of small parenchymatous cells.   These parenchymatous
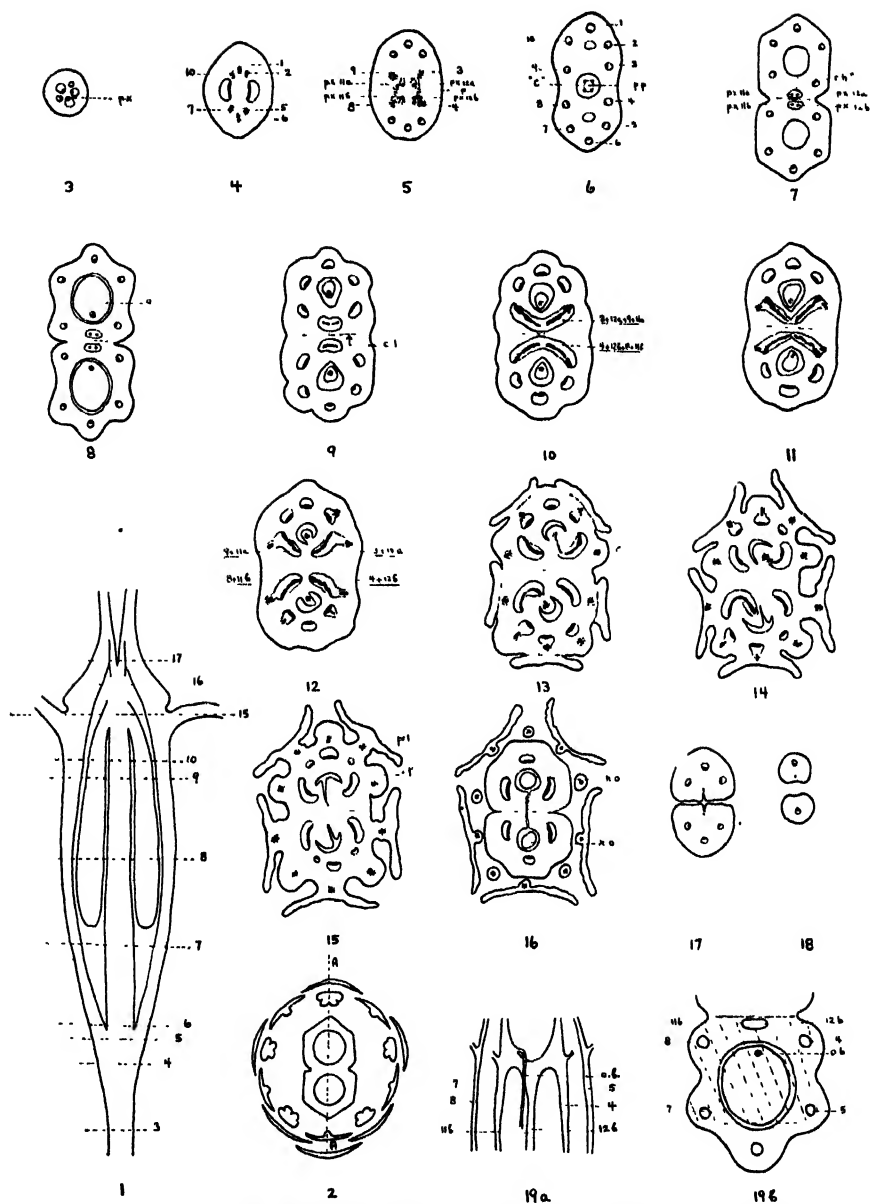
TEXT FIGS. 1–19. *Osmorhiza longistylis.* 1, longitudinal diagram of the flower through plane *A–A* of fig. 2, showing approximate levels of cross-section diagrams; 2, floral diagram; 3–18, cross-section diagrams of the flower at successive levels: 3–10 at levels 3–10 of figure 1, 11–14 at successive levels between levels 10 and 15 of figure 1, 15–17 at levels 15, 16 and 17 of figure 1; 19*a*, longitudinal diagram of the portion of the vascular system of the flower indicated in figure 19*b*. Only a short vertical extent is shown. The vascular system laid out in one plane and the ovule bundle displaced. *p.x.*, protoxylem; *p.p.*, pith-like parenchyma; *c.l.*, commissural line; *o*, ovule; *r.o.*, rudimentary ovule; "*c*," "carpophore"; "*c.h.*," "carpophore half"; *pet.*, petal; *st.*, stamen; *o.b.*, ovule bundle. (The bundles are numbered in the same order and manner throughout this paper.)

areas look much like the phloem areas of ordinary collateral or amphicribral bundles.   The parenchymatous tissue associated with the four central protoxylem strands is apparently like that of the main bundles.   It is not possible in the flower to determine by histological study how much of this tissue is young phloem and how much is parenchymatous tissue of other nature.   From evidence which will be presented later it is apparent that these parenchymatous areas represent partly phloem and partly some other tissue.

The parenchymatous tissue associated with the four central protoxylem strands unites as the strands move still farther in towards the center of the flower.   There is formed, centrally, a rod-like structure composed of this parenchymatous tissue, the four protoxylem strands, and some large-celled pith-like tissue included in the center (fig. 6).   The structure which has thus been differentiated in the center of the flower is what would be called the "carpophore" in the flower stage.   (The term carpophore is necessarily used somewhat loosely in the descriptions of material of different ages. The carpophore is a structure of the mature fruit and its true limits are not always apparent in material of flowers and young fruits.)   The central, pith-like parenchyma of the "carpophore" is gradually replaced by smaller-celled parenchyma which does not differ in appearance from the parenchymatous tissue associated with the four central propoxylem strands.

The "carpophore" soon becomes constricted into halves, the plane of constriction being parallel to the commissure.   This constriction is effected by a change in cell type and arrangement through the commissural region. Each of the "carpophore halves" consists of an area of small parenchymatous cells and the two small protoxylem strands which are situated close together, within the parenchymatous tissue (fig. 7).

Somewhat above the level of bifurcation of the "carpophore" there appear in the loculi the lowest parts of the two pendulous ovules, one in each loculus.   (There are present in each loculus of a young umbellifer ovary two anatropous ovules; one of these is erect and usually aborts, the other is pendulous and develops to maturity.)

Through the central part, vertically, of the flower, there is no change in the vascular plan.   Figure 8 shows the arrangement at a central level.

In the upper part of the flower, the ten main bundles and the "carpophore halves" become stronger (fig. 9).   The two protoxylem strands in each "carpophore half" are very close together or, often, are united into one xylem band (fig. 9).   The tissue of each of the "carpophore halves" unites with that of the two adjacent "laterals" [1] (figs. 10 and 19).   The $12a$ end of a "carpophore half" anastomoses with bundle 3 and the $11a$ end of the same "carpophore half" with bundle 9; the $12b$ end of the other "carpophore

[1] The term "laterals" is used here, and subsequently, to designate bundles 3, 4, 8 and 9.   This term is used merely for convenience and does not indicate that the bundles referred to are actually the lateral, ventral, bundles of the carpels in which they occur. As will appear later, they are not.

half" anastomoses with bundle 4 and the 11*b* end with bundle 8 (figs. 10 and 19). The anastomosis masses thus formed are designated *3 + 12a + 9 + 11a*, and *4 + 12b + 8 + 11b* (fig. 10). Each of these V-shaped anastomosis masses becomes constricted in the angle of the V (fig. 11) and separates into two parts (fig. 12). After this separation the four parts formed are referred to as *3 + 12a, 9 + 11a, 4 + 12b* and *8 + 11b* (fig. 12). A short distance farther up the ovule bundles become attached to the inner edges of anastomosis bundles *3 + 12a* and *8 + 11b* (figs. 13–14 and 19).

It is clear that the ovule bundles originate from the portions of these anastomosis bundles which came from the "carpophore halves." In some cases, the protoxylem of the ovule bundles appears to be directly continuous with protoxylem which runs up through the "carpophore halves."

A short distance above the anastomosis of the "carpophore halves" with the "laterals," a single small branch separates from the ends of each anastomosis mass (figs. 11–13). Within a few sections bundles nos. 1–2, 5–7 and 10 give rise to similar branches. These ten branches pass outward into the petals and stamens. The branches of bundles *2, 4 + 12b*, 6, *8 + 11b* and 10 supply the five petals. Those from bundles *1, 3 + 12a*, 5, 7 and *9 + 11a* supply the five stamens. No supply to the calyx was observed. (The calyx teeth in *Osmorhiza longistylis* are obsolete.)

After the separation from bundles 2, 5, 7 and 10 of the branches which supply petals and stamens, the remaining tissue of each of these bundles unites with adjacent bundles, gradually dies out, or often part of it unites with adjacent bundles and part dies out. Such variation as occurs is of minor importance.

There are left, thus, in the upper part of the ovary (level 16 of fig. 1) six bundles, three in each half (fig. 16). These six bundles pass outward into the two styles, three going into each style (fig. 17). The three bundles in each style become weaker as they pass on up and in the upper part of the style they become reduced to one (fig. 18). This reduction in number is usually effected by the fusion of all three of the bundles. Sometimes one of the lateral bundles (of the three) dies out gradually and the remaining two fuse.

In the upper part of the ovary, at a level above the insertion of the petals and stamens, the two loculi become interconnected (fig. 16). The passageway between the loculi is extremely narrow. It persists for only a few sections. (Similar openings between the two loculi of the ovary were found in many of the other members of the family examined.)

Partly Mature Fruit. The pedicel of the partly-mature fruit (fig. 20) shows a great deal more lignified tissue than that of the flower. Meristematic tissue of the flower (the parenchymatous areas of the bundles and other undifferentiated tissue of the flower pedicel) has developed into more mature tissue much of which is lignified. Each of the several bundles of the pedicel (fig. 20) shows a protoxylem area with considerable protoxylem
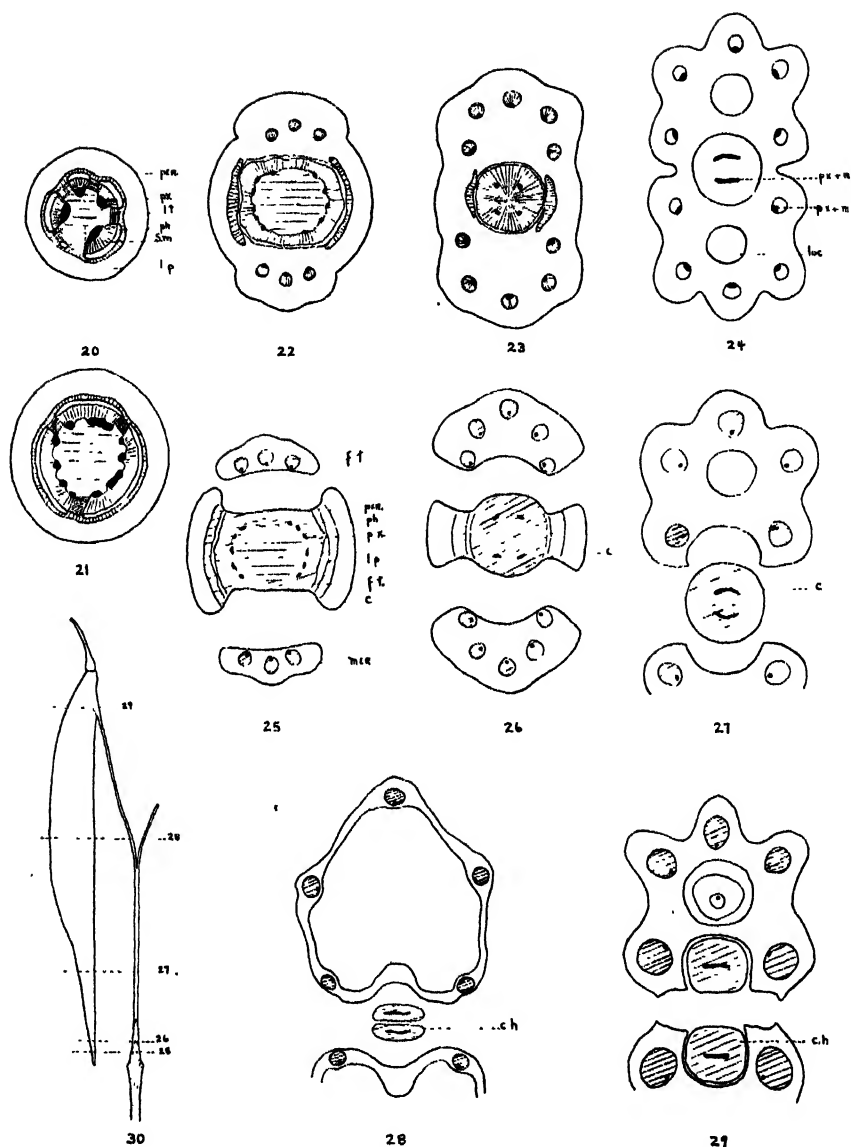
TEXT FIGS. 20–30. *Osmorhiza longistylis.* 20–24, cross-section diagrams of the partly-mature fruit at successive levels; 25–29, cross-section diagrams of the mature fruit at successive levels (these levels are indicated in figure 30, *e.g.*, fig. 25 is at level 25 of fig. 30); 30, diagram of longitudinal view of the mature fruit shown in a plane at right angles to the commissural plane. One mericarp and part of one carpophore half omitted. Figs. 25–27 are at approximately the same levels as figs. 22–24. The contents of the loculi are omitted in fig. 28. *l.t.,* lignified tissue between bundles; *s.m.,* specialized metaxylem, including typical conducting metaxylem (see text); *m.,* typical conducting metaxylem; *ph.,* phloem; *per.,* pericyclic region; *l.p.,* lignified pith; *loc.,* loculus; *mer.,* mericarp; *c.,* carpophore; *c.h.,* carpophore half; *p.x.,* protoxylem; *f.t.,* fibrous tissue.

parenchyma and, external to this, a metaxylem area composed chiefly of cells with thick, heavily lignified walls and protoplasmic contents. These are a specialized type of metaxylem cell. Scattered among them, near the protoxylem, are a few typical conducting metaxylem cells. Outside the metaxylem is a parenchymatous phloem area, and outside this a pericyclic region of sclerenchymatous cells. The tissue between the bundles is composed of thick-walled, lignified cells and the pith cells are also more or less lignified.

As the pedicel widens out, the vascular tissue increases in amount and forms a more nearly complete cylinder (fig. 21). From this cylinder the ten peripheral bundles pass out in the same manner as in the flower. As the first six of the bundles move out the pericyclic sclerenchyma disappears on the two sides from which they come. This causes the cylinder to look flattened on those two sides (fig. 22). Somewhat farther up the other four of the ten peripherals become differentiated and move gradually outwards, also with an accompanying loss of pericyclic sclerenchyma.

The remaining stelar tissue forms an irregularly rounded central mass which shows four protoxylem groups (fig. 23). These four protoxylem groups are differentiated in most cases as the last four of the peripherals pass outward from the stele. In some cases they become differentiated slightly higher up, that is after the last peripherals have passed out from the stele. The central structure, or "carpophore," consists largely of lignified tissue. The outer part, roughly that part outside of a line drawn through the inner margins of the four protoxylem groups (fig. 23), is metaxylem of the type described in the discussion of the pedicel. The central part is of more or less lignified parenchyma. This central lignified parenchyma is similar in appearance to the specialized metaxylem. Most of it is slightly larger-celled than that tissue, but the two tissues grade into each other. Around the outside of this central rod of lignified tissue is a thin area of non-lignified, parenchymatous phloem. On two sides of the "carpophore" there remain bands of pericyclic sclerenchyma. The histological nature of the "carpophore" tissue (aside from that of the central part and the remaining pericyclic sclerenchyma) is the same as that of the ten peripheral bundles.

In successively higher sections the central lignified parenchyma of the "carpophore" comes to appear more and more like the specialized metaxylem. It becomes histologically indistinguishable from that tissue, though of course it is still morphologically distinct.

A section through the lower part of the loculi (fig. 24) shows several changes. The four protoxylem and conducting metaxylem groups have united in pairs and form two bands of xylem which lie on opposite sides of the commissural line and parallel to that line. The tissue of the "carpophore" and that of the ten peripherals, except for the protoxylem and conducting metaxylem groups, is all thin-walled and non-lignified. This

is the condition throughout the main part of the fruit. The bands of pericyclic sclerenchyma have died out.

Above the bifurcation of the "carpophore," each of the "carpophore halves" consists of a band of lignified protoxylem and conducting metaxylem surrounded by thin-walled parenchymatous tissue. In the upper part of the fruit, just below the anastomosis level, the "carpophore halves" and the ten peripherals again consist almost entirely of thick-walled lignified cells like those described in the base of the fruit. Aside from this histological difference, the anastomosis of the "carpophore halves" with the "laterals" and the origin of the ovule bundles appear as in the flower.

Mature Fruit. Figures 25 and 26 show the line of separation of the mericarps (deciduous fruit "halves") in the lower part of the fruit. The histological structure differs from that described in the corresponding parts of the partly mature fruit chiefly in that here the metaxylem consists almost entirely of thick-walled fibers—apparently the mature state of the specialized metaxylem cells described in the immature fruit. Most of the other lignified tissue described in the immature fruit has also become fiber-like. At the level shown in figure 26 the central part of the carpophore consists of fibrous tissue which is not distinguishable from the fibrous metaxylem of the outer part.

Figure 27 shows the carpophore as a single rod-shaped structure composed chiefly of fibers. Figure 28 is of a section above the level of bifurcation of the carpophore. These two figures (27 and 28) represent the structure which is present throughout by far the greater part of the fruit length (fig. 30). In the upper part of the fruit the free carpophore halves become larger and less flattened (fig. 29). A short distance farther up they become attached to the mericarps.

Subfamily Apioideae. Tribe Ammineae
Example 1, Zizia aurea

The vascular tissue of the widened receptacular stele which does not become the ten peripherals moves inward toward the center and there forms four separate bundles (fig. 46). Each of these bundles consists of a tiny protoxylem strand (usually of a single protoxylem cell) and a group of small parenchymatous cells. Between the bundles is large-celled parenchyma. The four central bundles move closer together. "Carpophore halves" are formed by the union of the two bundles on each side of the commissural line and a small amount of parenchyma. The protoxylem strands do not unite (fig. 47). The parenchyma which is included in the "carpophore halves" becomes histologically indistinguishable from the parenchymatous tissue of the bundles.

The anastomosis of the "carpophore halves" with the "laterals" is similar to that in *Osmorhiza longistylis*. The attachment of the ovule bundles shows variation. In some cases the ovule bundles attach to the
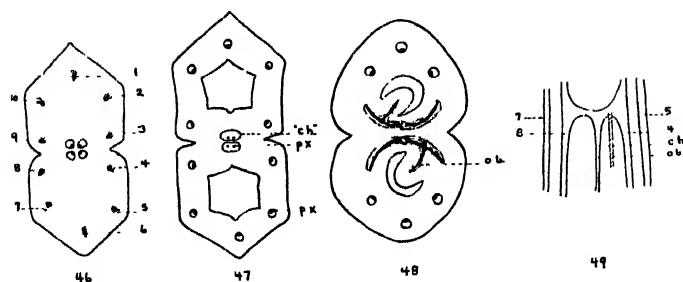
TEXT FIGS. 46–49. (Figures 31–45 are omitted.) *Zizia aurea*. 46–48, cross-section diagrams of the flower. Explanation in the text; 49, longitudinal diagram of a portion of the vascular system of the flower. This diagram shows the same part of the vascular system as is shown in figure 19*a* of *Osmorhiza longistylis*. *p.x.*, protoxylem; *o.b.*, ovule bundle; "*c.h.*," "carpophore half."

two anastomosis masses just after those masses separate into two parts each. This is essentially the same as in *Osmorhiza longistylis;* the level of attachment is merely a little lower. In other cases the ovule bundles attach to the anastomosis masses at the level of their formation or slightly above, that is, before the anastomosis masses break into two parts each (figs. 48–49). This variation is apparently of no fundamental importance as the two types are rather commonly found in the same flower. Furthermore the two types show intermediates. The origin of the ovule bundles from the "carpophore half" tissue is less obvious, in general, than in *Osmorhiza longistylis*.

Example 2, Aegopodium podagraria

Flower Stage. The receptacular stele breaks up into ten peripherals and two central bundles (fig. 50). Each of the bundles consists of a protoxylem strand and some parenchymatous vascular tissue. The two central bundles move closer to each other and with the parenchyma between them form a rod-like central structure (fig. 51). The parenchyma between the two
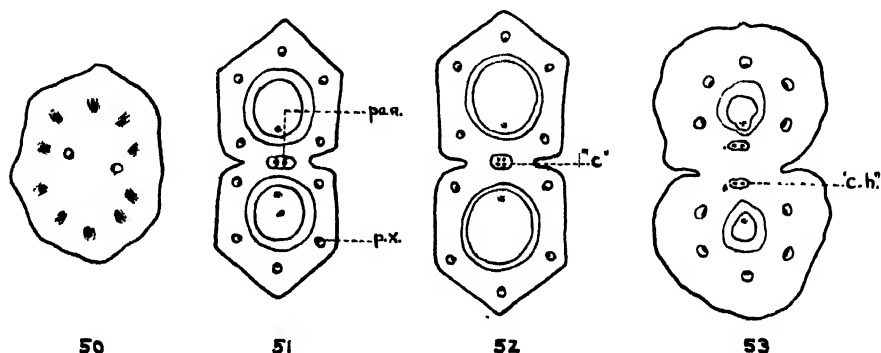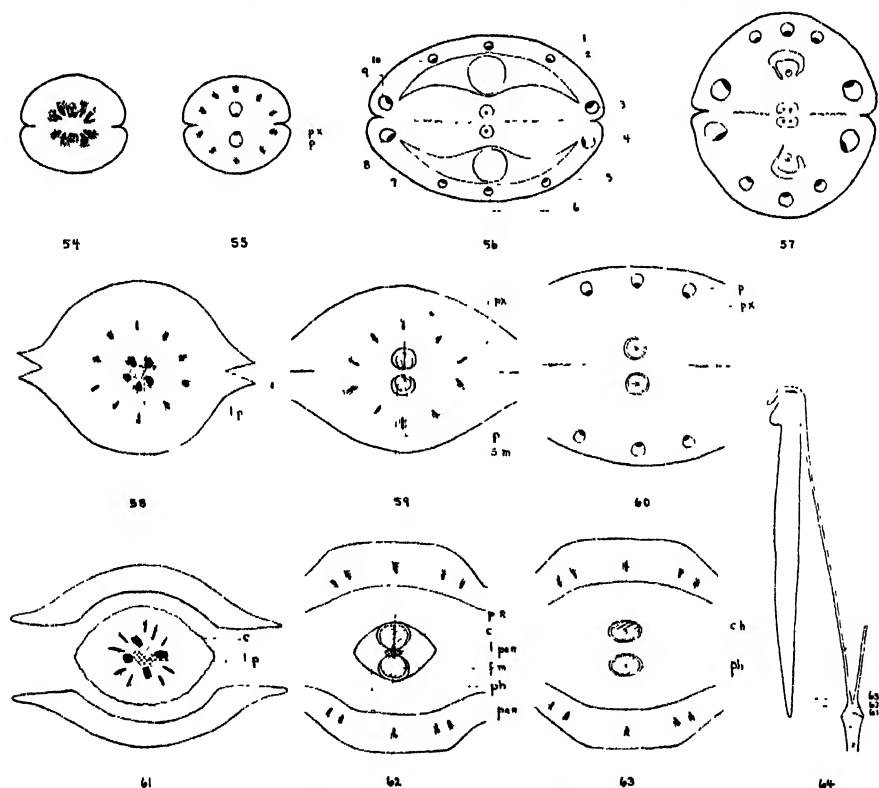

TEXT FIGS. 50–53. *Aegopodium podagraria*. Cross-section diagrams of the flower at successive levels. Explanation in the text. *p.x.*, protoxylem; "*c.*," "carpophore"; "*c.h.*," "carpophore half"; *par.*, parenchyma.

12

central bundles soon ceases to be distinct from the bundle tissue. Some-
what higher up, the two protoxylem strands in the "carpophore" split into
two parts each (fig. 52). This split occurs in a plane parallel to the com-
missural plane. In the upper part of the flower the "carpophore" splits
into halves in the same plane (fig. 53). The structure in other respects is
not fundamentally dissimilar from that described in *Zizia aurea*.

Subfamily *Apioideae*.   Tribe *Peucedaneae*
Example, *Pastinaca sativa*

Flower Stage. The tissue of the receptacular stele gives rise to ten
peripheral bundles and two central bundles (figs. 54–55). The two central
bundles very soon appear concentric. They remain separate from each

TEXT FIGS. 54–64. *Pastinaca sativa.* 54–57, cross-section diagrams of the flower at
successive levels. Explanation in the text; 58–60, cross-section diagrams of the partly-
mature fruit at successive levels through the lower part. Explanation in the text; 61–63,
cross-section diagrams of the mature fruit at the levels indicated on fig. 64. These levels
are approximately the same as those of figs. 58–60. Explanation in the text; 64, diagram
of longitudinal view of the mature fruit shown in a plane at right angles to the commissural
plane. One mericarp and part of one carpophore half omitted. *p.x.*, protoxylem; *p.*,
parenchymatous vascular tissue; *l.p.*, lignified pith; *s.m.*, specialized metaxylem, including
typical conducting metaxylem (see text); *c.*, carpophore; *p.r.*, protoxylem region; *l. par.*,
lignified parenchyma; *f.m.*, metaxylem, largely fibers; *ph.*, phloem; *c.h.*, carpophore half.

other throughout the flower. Figure 56 shows the structure at a central level. In the upper part of the flower, the xylem strand in each of the two central bundles splits into two parts in a plane at right angles to the commissural plane (fig. 57). Slightly farther up there is anastomosis of the central bundles with the "laterals" and attachment of the ovule bundles in a manner not fundamentally different from that described in *Zizia aurea*.

Young Fruit. This is essentially like the flower.

Partly Mature Fruit. The stelar tissue remaining after the ten main bundles pass out forms two central bundles (figs. 58-59). As in *Osmorhiza longistylis*, the appearance of this region is quite different from that of the corresponding region in the flower because of the presence of more mature stelar tissue. The two central bundles are apparently merely vascular bundles. They do not unite to form a central rod. They are separate throughout, as in the flower. Each bundle consists of a lignified mass and some small-celled parenchymatous tissue outside the lignified mass except between the bundles. The lignified mass consists of some typical conducting xylem, including protoxylem, and a much larger number of cells with lignified walls and protoplasmic contents which are similar to the specialized metaxylem cells described in the partly-mature fruit of *Osmorhiza longistylis*. The region between the two bundles is of large-celled parenchyma. Each of the peripheral bundles consists of a protoxylem strand and an area of parenchymatous tissue. The central bundles soon appear as shown in figure 60. This is the condition throughout the main part of the fruit.

Mature Fruit. The line separating the mericarps from the carpophore begins at the level of the break-up of the receptacular stele. The lowest parts of the free mericarps have no vascular bundles in them. The carpophore shows the breaking-up receptacular stele (fig. 61). Figure 62 shows a cross section slightly higher up. The peripheral bundles are now in the mericarps; the separation has taken place across them. The remaining stelar tissue has formed the two central bundles. The carpophore consists of these bundles, some large-celled, lignified parenchyma between them and some adjacent non-lignified parenchyma. The metaxylem of the central bundles now consists largely of fibers. Each of the peripheral bundles consists of protoxylem, possibly some conducting metaxylem, and some parenchymatous tissue. The non-lignified parenchyma of the carpophore soon dies out. A break occurs through the lignified parenchyma between the central bundles. Thus carpophore halves are formed which are merely the two central bundles. These soon appear as shown in figure 63. This is the condition throughout the main part of the fruit.

Subfamily Apioideae. Tribe Dauceae

Example, Daucus carota

Flower Stage. The tissue of the flower is all very immature. The vascular tissue of the receptacular stele which does not pass out as the ten

peripherals moves toward the center of the flower. This tissue is meristematic. The central structure will be described further in older material.

Partly Mature Fruit. The residual vascular tissue forms two central bundles. These two bundles and the large-celled parenchyma between them form a rounded central structure (fig. 65). Around this "carpophore"
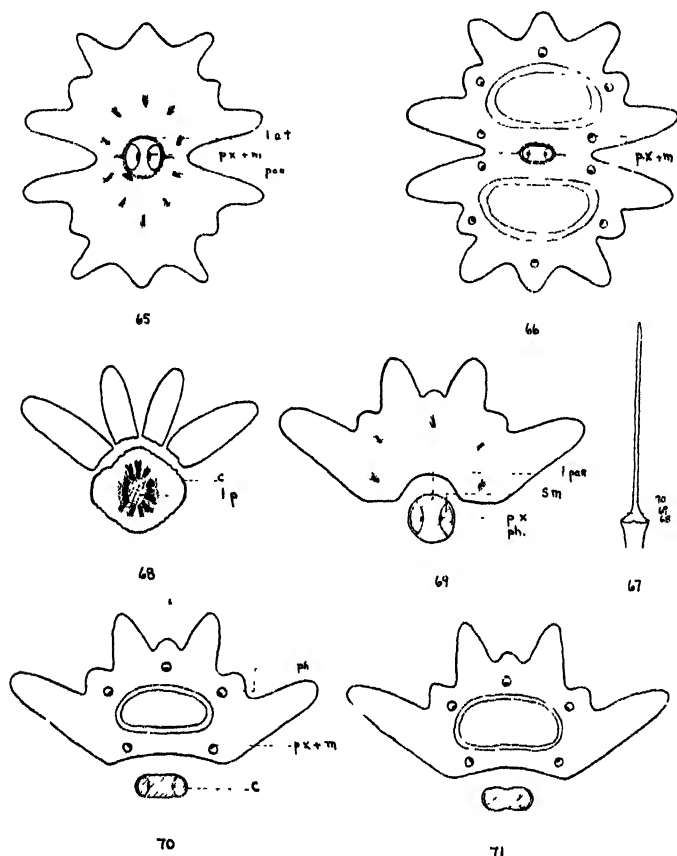
TEXT FIGS. 65-71. *Daucus carota.* 65-66, cross-section diagrams of the partly-mature fruit. Explanation in the text; 67, longitudinal view of the carpophore of a mature fruit shown in a plane at right angles to the commissural plane (the mericarps not shown); 68-70, cross-section diagrams of the mature fruit at levels 68-70 of fig. 67. The lower mericarp is omitted in figs. 68-70; 71, diagram of a section showing a carpophore with no protoxylem or conducting xylem. *p.x.*, protoxylem; *m.*, typical conducting metaxylem; *par.*, parenchyma; *l.a.t.*, loosely arranged tissue; *l.p.*, lignified pith; *c.*, carpophore; *l.par.*, lignified parenchyma; *s.m.*, specialized metaxylem, including typical conducting metaxylem (see text); *ph.*, phloem.

is an area of very loosely arranged tissue. Slightly higher up the large-celled parenchyma of the "carpophore" is replaced by smaller-celled parenchyma. The "carpophore" becomes smaller and somewhat flattened parallel to the commissural plane (fig. 66). It appears about as shown in this figure throughout the main part of the fruit. In the upper part the

"carpophore" rounds out again. At the anastomosis level it apparently splits parallel to the commissure as anastomosis with the "laterals" takes place. The ovule supply is derived from the anastomosis bundles.

Mature Fruit. The mericarps do not adhere to the carpophore; they fall off readily. The free carpophore is a strap-shaped structure which is not forked in a plane parallel to the commissural plane as is the carpophore in *Osmorhiza longistylis*, *Coriandrum sativum* and *Pastinaca sativa* (compare fig. 67 with figs. 30 and 64).

The mericarps become separated from the carpophore at the level of break-up of the receptacular stele (fig. 68). A section slightly higher up shows the carpophore as a rounded structure (fig. 69) which consists of the two central bundles and considerable connecting parenchyma which is lignified. Although shown so in the figures, the inner limits of the central bundles are actually not very clear. The limits can be judged fairly well from the position of the protoxylem. Each of the central bundles consists of a protoxylem region, a few conducting metaxylem cells, some lignified cells like the specialized metaxylem described in other forms and, outside this, some small-celled parenchymatous tissue, apparently phloem. The lignified parenchyma of the carpophore grades into the tissue of the bundles.

Slightly higher up, the carpophore becomes smaller and somewhat flattened (fig. 70). Most of its tissue is more or less lignified. The exact limits of the bundles are not obvious. This is the approximate appearance of the carpophore throughout its main part. Near the top it rounds out again.

Variations. The above description is based on what, in the opinion of the author, is the typical, or primitive, condition in *Daucus carota*. Not all of the material examined showed the structure described. In some material no protoxylem or other conducting xylem is present in the central bundles. Either no such xylem strands pass into the carpophore at its origin or those which do pass into it die out at once. In other respects the structure of the carpophore is essentially the same as has been described. Figure 71 shows a section through the central part of such a carpophore. When no protoxylem strands are present the limits of the central bundles are not discernible. The bundles are indicated in the figure by dotted lines.

In still other material examined the carpophore shows one protoxylem strand. This strand is in the position of either of the two in those carpophores which show two (*e.g.*, fig. 70). Obviously, one of the central bundles is less reduced than the other in such material.

Subfamily Hydrocotyloideae.   Tribe Hydrocotyleae
Example, Hydrocotyle americana

Flower Stage. At the break-up of the receptacular stele, ten bundles pass outwards towards the periphery. As this takes place, there pass into the center, or appear in the central region, one or more delicate protoxylem cells. Associated with these is a certain amount of highly protoplasmic

stelar parenchyma. These delicate protoxylem cells and the accompanying parenchyma die out within a few sections.

About two-thirds of the way up, each of the "laterals" branches tangentially into two parts. Somewhat higher up each of the four inner parts sends a branch toward the center of the ovary. The two of these four branches in each flower-half unite and become the ovule bundle.

Somewhat higher up, the other six of the ten peripherals branch tangentially. There are then ten outer bundles and ten inner bundles present. The ten outer bundles supply the petals and stamens. Of the ten inner bundles, the five in each flower-half unite and run up into the style.

Fruit Stages. These offer no additional information of importance in the present study.   .

Other Members of the Subfamily. Material of other species which was examined showed nothing additional of importance. Some members of this subfamily are reported to have a carpophore. None of the material examined showed this.

Subfamily Saniculoideae. Tribe Saniculeae
Example, Eryngium amethystinum

Flower Stage. At the break-up of the stele, ten peripherals are formed. No central strands were observed (fig. 72).

About half way up, four delicate vascular bundles appear in the position of bundles *A*, *B*, *C* and *D* of figure 73. These four bundles do not all appear at the same level. First one is evident, then another, and so on. They are very small bundles. Each consists, usually, of a single lignified xylem cell and a small amount of associated parenchyma. Throughout the lower parts of these tiny bundles their lignified xylem elements die out, off and on, and then reappear. All four tiny bundles were not observed in all the flowers examined. Some showed only one or two. Young flowers showed none.

In the upper part of the flower, the four central strands become stronger. Branches from the inner edges of the "laterals" move in toward the center (fig. 74). These branches anastomose with the four central strands (fig. 75). The parenchymatous parts of the two centrals in each anastomosis mass are united (fig. 75). The anastomosis masses separate into two parts each and the ovule bundles become attached to two of the four bundles thus formed (fig. 76). These four bundles (fig. 77) then pass up into the styles, two into each style.

The rest of the bundles in the ovary soon form an arc of vascular tissue in each flower-half. From these two vascular arcs there separate bundles to the sepals, petals and stamens. Each of these parts gets one bundle. Most of the tissue of the vascular arcs is used up thus. The rest, in the form of a number of small, irregular bundles, passes inward through the tissue of the stylopodia.

Fruit Stages. These show the same anatomical plan as the flower. The four small central strands do not have any connection at the base with the stelar tissue. The histological structure is complex. Descriptions of the fruit stages are therefore omitted since their inclusion seems unnecessary.

Other Members of the Subfamily. In the material which was examined the structure of the central region was in general similar to that in *Eryngium amethystinum*, only much more reduced. The central strands were not as a rule seen in flowers but were evident, usually, in fruit stages.
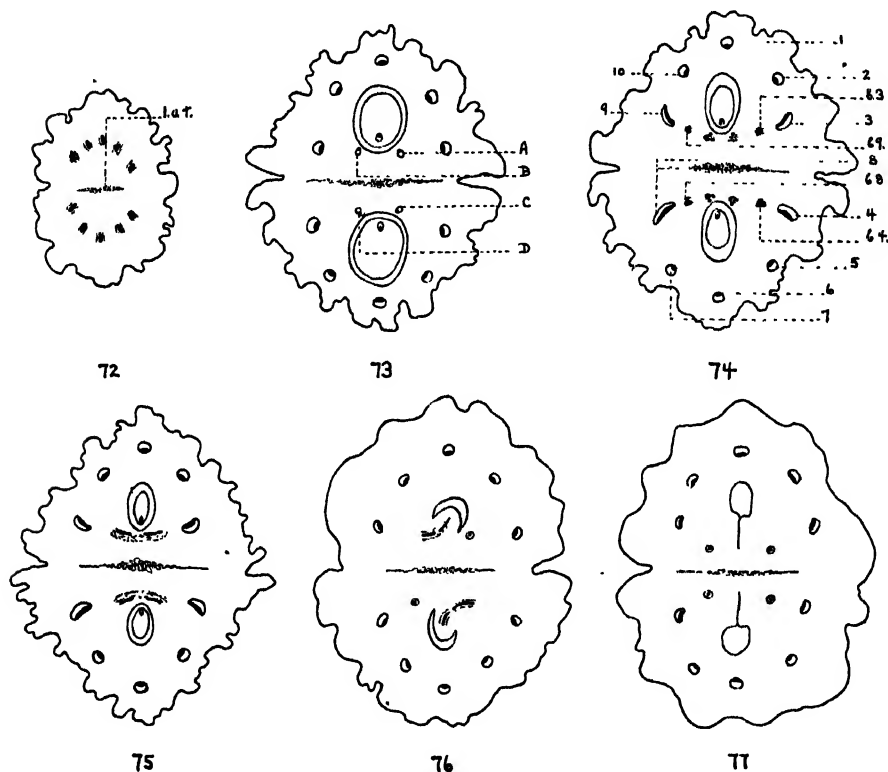
TEXT FIGS. 72–77. *Eryngium amethystinum.* Cross-section diagrams of the flower at successive levels. Explanation in the text. *b.3.*, branch of bundle 3; *b.4.*, branch of bundle 4; etc.; *l.a.t.*, loosely arranged tissue of commissural line.

In those cases in which the centrals are not evident at all, branches from the "laterals" form anastomoses which are in general appearance similar to those in figure 74, and which act the same as those.

<center>DISCUSSION</center>

Composition of the carpophore

From the descriptions given above of the vascular structure of the flowers and fruits of various genera of the Apioideae it is seen that the structure

called the carpophore is composed largely of vascular tissue. Associated with this vascular tissue is some non-vascular tissue. The amount of the latter varies in the different forms described and in the different parts of the carpophore.

It must not be overlooked that it is difficult to draw a sharp line between vascular and non-vascular tissues in a flower. The phloem of the bundles of most flowers consists largely or wholly of parenchyma and therefore often grades into surrounding non-vascular parenchyma. The approximate limits of the bundles in a flower are reasonably clear, but one cannot be categorical in stating exact limits. In the case of fruit stages the same indefiniteness usually occurs. Sclerenchymatous tissue of vascular origin frequently grades into and is indistinguishable from similar tissue of non-vascular origin. For this reason only the approximate amount of non-vascular tissue which is included in the carpophore can be determined.

It is necessary at this point to discuss what is meant by the term carpophore, that is, at what level the carpophore begins and the receptacle ends. Obviously there is no sharp line between the two. As far as the present author has been able to determine, the carpophore is considered to begin at the level at which the mericarps become free in the ripe fruit (figs. 30, 64 and 67). From anatomical study it is apparent that the mericarps often become free at a level at which the receptacular stele is just breaking up, that is, before the ten peripherals have passed out (figs. 25, 61 and 68). The mericarps thus often extend below the fruit proper into the receptacle. Nevertheless, it seems advisable to consider that the carpophore begins at the level at which the mericarps become free, because this is the only limit apparent to ordinary observation (i.e., with the naked eye or with a hand lens). The lowest part of a carpophore thus delimited is often (probably usually) definitely receptacular in nature, because traces to floral organs (the ten peripherals) have not as yet been given off, e.g., Osmorhiza longistylis, Pastinaca sativa, Daucus carota. This obviously receptacular portion of the carpophore is of very short vertical extent. By far the greater part of the carpophore is of a different morphological nature as is apparent from the present study.

After the ten peripheral bundles have passed out from the receptacular stele the residual vascular tissue[2] and some non-vascular tissue form some sort of central structure which is usually two-parted for a considerable portion of its length. Typically more non-vascular tissue is included in this central structure in its lower part than in the upper part. This variable central structure becomes in the ripe fruit the carpophore, or at least forms the greatest part of it.

It seems evident, from the information obtained in the present comparative study, that the vascular tissue of which this central structure

[2] The term vascular tissue is used in this paper to signify tissue of vascular origin whether it be conducting or not. Non-vascular tissue is tissue of non-vascular origin.

largely consists represents, typically, four vascular bundles. In support of this are the following observations:

1. The formation in some genera of four separate central bundles in the flower, *e.g.*, *Zizia aurea* and other forms examined but not described.

2. The constant occurrence of four protoxylem groups (although the parenchymatous vascular tissue is not in four units) in the flower of *Osmorhiza longistylis*.

3. In many forms in which two central bundles are formed at the base (*Aegopodium podagraria*, *Pastinaca sativa* and others), the splitting of the two protoxylem groups of these bundles to form four. This splitting indicates, in the opinion of the writer, that in the forms which show two central bundles each of the two bundles represents two of the four primitively present, which have become fused. (This splitting varies with respect to the level at which it occurs and the plane in which it takes place.)

In forms in which the carpophore shows no apparently conducting xylem cells through most of its extent, *e.g.*, *Conium maculatum*, and consists of parenchymatous tissue in the young stages and of fiber-like tissue in the mature stage, this tissue is considered to be modified vascular tissue. That there are four bundles involved is indicated by the similarity of the general anatomical structure to that in those forms which have four bundles or a more evident modification of them.

The carpophore consists then, in its main part, of four vascular bundles, present in various degrees of development, plus a variable amount of non-vascular tissue. Each of the halves of the split carpophore consists of, or represents, two vascular bundles with some adjacent non-vascular tissue, or in some cases apparently two vascular bundles alone.

The only evidence of a central structure in *Hydrocotyle americana* is the presence of the delicate central vascular strands at the level of the breaking-up of the receptacular stele. In the opinion of the writer, these strands are vestigial stelar prolongations. This tissue is comparable to the tissue in the Apioideae which is not reduced and which becomes differentiated as the central, or carpophore, bundles.

The other species of *Hydrocotyle* which were examined showed nothing additional of importance in the present study. Certain other members of this subfamily, which, unfortunately, were not available for the present study, are reported to have a carpophore.

No central strands were observed at the level of break-up of the stele in the members of the Saniculoideae which were examined. In the opinion of the writer, the small central strands described in the flower of *Eryngium amethystinum* are vestigial, and represent the four central bundles which are considered to be typically present in the Apioideae. That this is so seems evident from the position, number, regularity and connections of these small bundles. These four bundles have become greatly reduced and have lost their lower portions.

Morphological nature of the ovary

It is desirable, next, to inquire into the morphological nature of the bundles which form by far the greater portion of the carpophore. It becomes, therefore, necessary to interpret the structure of the ovary through which these bundles run.

Studies in the comparative morphology of floral parts, based on their anatomical structure, have shown that the wall of the inferior ovary consists, in the great majority of cases at least, of the basal portions of all the parts of the flower fused and indistinguishable one from the other.

There accompanies this fusion of floral organs, usually, a fusion of the traces to those organs. Those traces which lie in the same radius unite, and thus there is formed a bundle which, though apparently simple, is really morphologically compound, representing a number of floral traces which have been fused in phylogenetic development.

In the umbellifer flower the sepal, petal, stamen, and carpel whorls are adnate at the base and for a considerable vertical extent, and those of their traces which lie in the same radii have also become fused. The ten peripheral bundles formed at the breaking-up of the receptacular stele (bundles 1–10 of the figures) are not simple traces but compound bundles composed of fused traces to more than one floral part.

Each of these bundles which is in the radius of a petal, (bundles 2, 4, 6, 8 and 10), is composed of the trace to a petal and one of the carpel traces. Each of the bundles in the stamen radii (bundles 1, 3, 5, 7, 9) consists of one of the carpel traces, the trace to a stamen, and at least in its lower part, the trace to the sepal in that radius.

Although the calyx teeth are very often obsolete and, therefore, sepal traces as such are, as a rule, not apparent, from a morphological standpoint the sepal traces compose part of the main bundles in the sepal radii, at least in the lower portion of the ovary. This will be intelligible from consideration of those forms in which traces to the calyx teeth are present.

In the upper part of the ovary, near the level of apparent insertion of sepals (calyx teeth, when present), petals and stamens, the traces to those parts become separate from the other traces with which they are fused— carpel traces—and pass out into their respective floral parts. The branches from the ten main bundles described in *Osmorhiza longistylis* are these traces.

In all the members of the Apioideae described and in *Eryngium* these traces become free from the carpel traces, to which they are fused, at approximately the same level—just slightly below the apparent insertion of sepals, petals and stamens, and pass almost directly out into those organs. In *Hydrocotyle americana* the traces to the petals and stamens separate from the carpel traces at levels considerably below the apparent insertion of the petals and stamens and run up through the ovary wall for some distance before passing out into those organs.

Vascular system of the carpels

The Apioideae. Those continuations of the ten main bundles which pass upwards above the level of separation of traces to sepals, petals and stamens, and usually enter the styles are carpel traces; five belonging to each carpel. The continuations of bundles 1 and 6 are the midrib bundles of the carpels. In addition to these five traces, each carpel in the Apioideae (the carpel supply in the two other subfamilies will be considered later) has two ventral traces. The total number of carpel traces is evidently seven.

From the anatomical structure it seems apparent that the four central bundles typically present in the Apioideae represent the four ventral bundles of the two carpels. That these four bundles are the ventral traces of the two carpels seems evident for the following reasons:

1. Their typical number, four, representing two traces from each carpel.

2. The regular and constant positions of the four central bundles or of the four protoxylem strands. Four bundles or four protoxylem strands in the center of the ovary, if representing four stelar bundles, might very well be arranged in some manner other than that shown in figures 5–6, 46–47 and 52. No other arrangement was observed in any case. The arrangement is always as shown, an arrangement which is that to be expected for the four ventral traces of the two carpels.

3. The obvious connection of the central bundles with the ovule supply which was observed in all the material examined. In all the members of the Apioideae examined, except *Coriandrum sativum*, there takes place, in the upper part of the ovary, anastomosis between the central bundles, which are at that level united in pairs, and the adjacent "laterals." The ovule bundles are derived from these anastomoses, and sometimes the ovule bundles clearly originate from tissue which is a direct continuation of the central (ventral) traces.

It would seem to the author that the evidence which has been reviewed indicates clearly that the central bundles are the ventral traces of the carpels.

These four ventral traces are present in the members of the subfamily in various conditions. In some cases, as has been shown, they appear, at least at some level in the flower, as four separate strands (*Zizia aurea*). In other members they are never present as four separate strands but are united, the method and the extent of the union being variable.

It may be of some value to consider briefly this fusion. In the carpels of a syncarpous angiosperm ovary, fusion of the two ventral traces of one carpel or of two ventrals, one from each of two adjacent carpels, is a rather commonly observed condition. This fusion of pairs of ventrals may even extend to their place of origin in the receptacle. It seems clear from comparative study that this is the condition in the ovaries of many of the Apioideae. Each of the two central bundles in types like *Pastinaca sativa*, *Aegopodium podagraria*, *Coriandrum sativum*, *Daucus carota*, represents two

fused ventral traces. The fusion is sometimes of the two ventrals of one carpel (*Pastinaca sativa*). In other cases the two ventrals of different carpels are united (*Coriandrum sativum, Aegopodium podagraria, Daucus carota*).

In *Osmorhiza longistylis*, all four of the ventrals are fused to a considerable extent at the base. The four protoxylem groups of the ventrals, however, remain separate. The carpophore half in this form represents two fused ventral traces and a certain amount of adjacent tissue, not distinguishable histologically from the bundle tissue. The carpophore half in *Zizia aurea* is of the same nature.

It is apparent, from the histological nature of the central bundles in various representatives of the Apioideae, that the physiological supply to the ovules is often largely through the laterals. It is possibly entirely so in those forms which show a much modified condition of the central bundles. On the other hand, in forms like *Osmorhiza longistylis*, in which the carpophore is large and shows considerable obvious conducting xylem throughout its extent, the physiological supply to the ovules is apparently largely through the tissue of the carpophore. Many of the forms examined show intermediate conditions.

The Hydrocotyloideae. The carpel supply differs from that in the Apioideae in the loss of the central bundles, ventral traces. The next most lateral bundles of the carpel have taken over completely the function of the ventrals. The ovule bundle is double in origin. It seems probable that the ovule supply here represents the bundles of two ovules formerly present. Both of the bundles for these two ovules now run into the single ovule which has become nearly median in position.

The Saniculoideae. The reduced central bundles in the upper part of the ovary of *Eryngium amethystinum* are considered to be the reduced ventral carpellary traces. Branches from the "laterals"—really the carpellary-trace portions of those compound bundles—anastomose with the reduced ventrals and from the resulting anastomosis bundles the ovule bundles are derived. The four anastomosis bundles continue up into the styles. The other carpellary traces (carpellary parts of bundles 1, 2, 10, 5, 6 and 7) are very much reduced in vertical extent. They are represented by those portions of the vascular arcs which pass into the stylopodia.

Morphological nature of the carpophore

The evidence obtained in the present study demonstrates clearly, in the opinion of the present writer, the true morphology of the carpophore: a small basal portion is usually receptacular or axial; by far the greater part is appendicular. It is a specialized fruit structure related to dehiscence and dissemination. It represents an innermost, ventral portion of the two carpels and consists chiefly of the ventral traces of these carpels. Associated with the traces in the formation of this structure is a greater or lesser

amount of adjacent non-vascular tissue. The development of a peculiar method of dissemination in an "indehiscent" fruit has resulted in the splitting of the ovary along unusual lines.

It seems desirable to consider here just where the limit between the axial or receptacular portion of the carpophore and the appendicular part lies. As has been stated in the descriptions, the residual stelar tissue is differentiated into the central bundles, ventral traces, or into the modified form in which the ventrals are found in the various members of this group, slightly above the level at which the ten peripheral bundles depart or at that level. It is at this level of differentiation of the ventral traces that the line between the axial and the appendicular parts of the carpophore lies, *e.g.*, between levels 25 and 26 of figure 30; between levels 61 and 62 of figure 64; between levels 68 and 69 of figure 67.

If the mericarps become free from the carpophore at a level below that of the differentiation of the ventral traces, the base of the carpophore is axial, as has been noted above. This is the condition observed in the material of fully ripe fruits examined in this study. Apparently this is the usual condition.

If, however, the mericarps become free from the carpophore at a level above the differentiation of the ventral strands, the entire carpophore is appendicular. This condition may very well exist in material which has not been examined in the mature condition.

Previous theories and investigations

As has been noted above, the prevalent statement concerning the nature of the carpophore is that it is axis-like or axial. Commonly, the exact meaning of the author's statement is not clear—that is, whether he considers the carpophore morphologically axial or merely an axis-like structure of unknown or at least unstated morphology. Some authors state definitely that the carpophore is the prolonged floral receptacle or floral axis (Gray and LeMaout and Decaisne); it is, therefore axial from a morphological standpoint in their opinion.

The present author has discovered no investigations which attempt to substantiate the axial theory; it is usually presented as a fact, and seems to be based on the external appearance of the carpophore in its free condition and upon its general appearance in sections, chiefly of the fruit.

As has been stated above, the evidence obtained in the present investigation shows clearly that the carpophore is almost entirely appendicular. Only a small basal portion is axial. The axial theory is, therefore, erroneous and should be modified to agree with the observed facts.

The appendicular theory of the morphology of the carpophore has been previously supported by investigations and evidence of anatomical nature similar to that brought forward in the present study.

Von Mohl studied transverse sections taken at different levels through

the partly-ripe fruits of a large number of representative genera and species, and compared them with longitudinal sections. From this study he concludes that the carpophore is not a separate structure distinct from the carpels, but that it is a definite part of the carpels which, in the ripe fruit, generally becomes free from the rest of the carpel tissue and then appears as a special organ. The carpophore consists, in his opinion, of the marginal (ventral) bundles of the carpels. His conclusions are, he says, at variance with the commonly existing theory that the carpophore is an elongation of the flower pedicel. He did not, apparently, study the entire vascular system, and he seems to have studied only partly-ripe fruits. Therefore, his evidence is much less convincing than would appear from his conclusions.

Van Tieghem, in discussing the fruit of the Umbelliferae, states that the filament from which the diakenes (mericarps) hang at maturity is nothing but the central portion of the ovary wall containing the marginal bundles of the carpels. He does not attempt to substantiate this statement.

Henslow, in a discussion on certain apparent but misleading axial formations in flowers and fruits, says that the carpophore on which the mericarps of an umbellifer fruit are supported is usually considered as axial, but that anatomical investigations do not warrant the conclusion. The vascular cylinder of the pedicel spreads out, he says, at the base of the inferior ovary, into twelve cords, ten of which supply the petals and stamens, while the other two coalesce and form the axial cord. It is this cord which constitutes the carpophore when the fruit is ripe. Hence it is not, in his opinion, axial, but is simply the combined marginal cords of the two ovary cells.

Henslow did not, apparently, study many different forms. His descriptions of the vascular structure, which is based on the few he did investigate, applies only in a general way to the family as a whole, which has many different types of structure in the central region.

The conclusions of von Mohl, Van Tieghem and Henslow agree in general with those of the present writer. A difference exists in that they consider the carpophore wholly appendicular whereas, as has been shown in the present study, a short basal portion of it is often definitely receptacular. This feature is evident only when sections of ripe fruits are examined.

SUMMARY

A study was made of the vascular structure of flowers and fruits of representative genera of the Umbelliferae with the hope of ascertaining the morphological nature of the carpophore. The information obtained from this study leads to the following conclusions.

The carpophore consists of the central part, or core, of the ovary. It is largely appendicular in nature. A short basal portion of it is usually definitely receptacular or axial.

The carpophore above this base consists of the ventral traces of the two carpels with a variable amount of adjacent, non-vascular tissue.

The carpophore is a peculiar fruit structure associated with the highly specialized type of fruit and of fruit dissemination found in the Umbelliferae.

The mericarps are also peculiar fruit structures. They consist largely or entirely of the tissues of an inferior ovary, and are, therefore, complex in morphological nature.

Those members of the family in which four separate ventral bundles are present (in at least some part of the flower or fruit) are considered to be, in that respect, more primitive than those in which these separate strands are not present. The presence of strong ventral strands in any form is considered a more primitive condition than the absence of them, or their presence only in a much reduced condition.

The ventral traces have become modified in various degrees in the development of the carpophore. This modification consists, fundamentally, in the development of a central structure which is essentially supporting. This specialization of the ventral traces has resulted, apparently, in a reduction of conducting function in some cases.

Accompanying this specialization of the ventral traces, the ovule bundles, which are primitively derived from the ventrals (the normal place of origin for ovule bundles), tend to get their physiological supply from the "laterals," the carpellary traces next in line.

The writer wishes to express her gratitude to Professor Arthur J. Eames of Cornell University for his helpful advice and kindly criticisms throughout the progress of this investigation.

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EFFECT OF EIGHTEEN NORMAL ALIPHATIC ALCOHOLS ON GROWTH OF *LUPINUS ALBUS*

DAVID I. MACHT AND JANE D. MEYER

INTRODUCTION

Comparative pharmacology and toxicology of various alcohols have been the subject of investigation on the part of many writers. Most of the work on this subject, however, has been confined to the first five members of the aliphatic series, namely, methyl, ethyl, propyl, butyl, and amyl alcohols and almost entirely to the primary alcohols in cases where several chemical isomers are known. The more important literature on the subject is quoted in a paper by one of the writers, which deals with the relative toxicity of some aliphatic alcohols with especial reference to their isomers (1920). In that investigation it was found that the first five members of the aliphatic series increase in toxicity with their molecular weight and also that the secondary are less toxic than the primary alcohols for animal tissues and whole animals. Practically all pharmacological work with alcohols has hitherto been of a zoöpharmacological nature; that is, studied on animal organisms. The effects of alcohols on living plants have been but little investigated. The present writers have made a comparative study of the pharmacology and toxicology of a long series of primary aliphatic alcohols on both zoöpharmacological and phytopharmacological test objects. A brief account of the results obtained with plants is presented herewith.

METHODS OF STUDY

The method employed was observation of the growth of seedlings of *Lupinus albus* in plant-physiological solutions containing definite percentages of the various drugs. Seeds of *Lupinus albus* were soaked in tap water overnight and planted in finely ground sphagnum moss. When seedlings thus germinated had developed roots from 30 to 45 mm. in length, they were ready for study. The length of the roots having been carefully measured, the seedlings were placed in upright test tubes of hard glass, containing solutions of equal parts of Shive medium (1915) and distilled water, with and without the alcohols to be studied. The plants were incubated in the dark at a temperature of from 20 to 21° C.; and at the end of twenty-four hours the increment in the straight, well-defined roots was carefully measured and compared, in each case, with that of the normal controls. Thus the growth of seedlings in solutions of various alcohols was expressed as a percentage of that of controls in normal physiological saline without alcohol.

145

13

To this coefficient or ratio the term *phytotoxic index* is applied.   Macht and Livingston have described the method employed in greater detail in a paper dealing with the effects of cocaine and its decomposition products on living seedlings (1922).

ALCOHOLS STUDIED

Eighteen different primary alcohols of the aliphatic series were studied in the present investigation.   These were prepared in highly purified form in the Laboratory of Organic Chemistry, Johns Hopkins University, under the direction of Professor E. Emmet Reid, to whom grateful acknowledgment is made.   Various concentrations of the alcohols were studied.   Since the solubility of the various alcohols rapidly decreases with their molecular weight, it was found most convenient to make the studies with concentrations of from 1 : 10,000 to 1 : 20,000.   Four of the alcohols, namely those containing from fifteen to eighteen carbon atoms, respectively, are solids at room temperature and but slightly soluble in water.   To study these a saturated solution was obtained by boiling small quantities of each in plant-physiological saline.   Such saturated solutions of the higher alcohols contain no more than one part in from 30,000 to 50,000 of the chemicals used. In making the studies, the average increment in growth of at least ten seedlings in each solution was usually determined, and such experiments were repeated many times so that the figures obtained for the phytotoxic indices represent the data derived from a very large number of plants, which are fairly accurate, especially in regard to expressing the relative toxicity of the various drugs examiṇed.

In addition to those made with primary or normal alcohols, a number of experiments were performed with several secondary and tertiary alcohols. Furthermore, the effect of mixtures or combinations of various members of the series, as compared with that of the individual alcohols, was also observed because of the extreme importance of synergistic phenomena in all pharmacological work.

RESULTS

The results obtained are exhibited in tables 1 and 2 and expressed in part by text figure 1.   In table 1 are given the phytotoxic indices of the eighteen primary alcohols of the aliphatic series.   The first two members of this series, namely, methyl and ethyl alcohols, are so very little toxic for the growth of plants that the concentrations employed had to be 1 : 1,000 in order to exert any influence on *Lupinus albus* seedlings.   The higher members of the series were employed in concentrations of 1 : 10,000 with the exception of the last four members, used as saturated solutions because of their very low solubility.   It will be noted that the toxicity of the first five members of the series increases progressively with the molecular weight of the alcohols.   Other investigators, experimenting with animal organisms, have noted this increase in toxicity or conformity to Richardson's law, so called in honor of the physician who first observed that relationship (1869).

TABLE 1. *Growth of Lupinus albus in Solutions of Normal Higher Alcohols at 21° C. in the Dark*

| Drug	Formula	Solution	Phytotoxic Index
Methyl alcohol	$CH_3 \cdot OH$	1 : 1,000	94%
Ethyl alcohol	$C_2H_5 \cdot OH$	1 : 1,000	87%
Propyl alcohol	$C_3H_7 \cdot OH$	1 : 10,000	71%
Butyl alcohol	$C_4H_9 \cdot OH$	1 : 10,000	63%
Pentyl alcohol	$C_5H_{11} \cdot OH$	1 : 10,000	46%
Hexyl alcohol	$C_6H_{13} \cdot OH$	1 : 10,000	88%
Heptyl alcohol	$C_7H_{15} \cdot OH$	1 : 10,000	94%
Octyl alcohol	$C_8H_{17} \cdot OH$	1 : 10,000	59%
Nonyl alcohol	$C_9H_{19} \cdot OH$	1 : 10,000	19%
Decyl alcohol	$C_{10}H_{21} \cdot OH$	1 : 10,000	10%
Undecyl alcohol	$C_{11}H_{23} \cdot OH$	1 : 10,000	44%
Dodecyl alcohol	$C_{12}H_{25} \cdot OH$	1 : 10,000	83%
Tridecyl alcohol	$C_{13}H_{27} \cdot OH$	1 : 10,000	96%
Tetradecyl alcohol	$C_{14}H_{29} \cdot OH$	1 : 10,000	94%
Pentadecyl alcohol	$C_{15}H_{31} \cdot OH$	Saturated	84%
Hexadecyl alcohol	$C_{16}H_{33} \cdot OH$	Saturated	92%
Heptadecyl alcohol	$C_{17}H_{35} \cdot OH$	Saturated	97%
Octodecyl alcohol	$C_{18}H_{37} \cdot OH$	Saturated	95%

TABLE 2. *Toxicity of Secondary and Tertiary Alcohols and Some Combinations for Lupinus albus*

Alcohols	Index of Growth in 1% Solution	Alcohol Mixtures	Index of Growth in 1% Solution	Alcohol Mixtures	Index of Growth in 1% Solution
Methyl	70%	Methyl and ethyl	80%	Fusel oil	5%
Ethyl	75%	Propyl and isopropyl	56%	Ethyl and fusel oil	17%
Propyl	38%	N-butyl and sec-butyl	19%		
Isopropyl	63%	Ethyl and propyl	82%	Ethyl—40 parts	
N-butyl	19%	Ethyl and butyl	20%	Methyl —4 parts	
Sec-butyl	27%	Ethyl and tert-amyl	70%	Propyl—2 parts	52%
N-amyl	0%	Ethyl and n-amyl	4%	Butyl—2 parts	
Sec-amyl	16%	N-amyl and sec-amyl	0%	Fusel—3 parts	
Tert-amyl	34%	Sec-amyl and tert-amyl	27%		

Earlier writers thought that the rule could be generally applied and that the toxicity of all alcohols increases with their molecular weight. As will be seen from table 1, however, this is not the case. It will be noted that although pentyl alcohol is very toxic for *Lupinus albus* seedlings, hexyl and heptyl alcohols are much less poisonous for the same plants. Beginning with octyl alcohol, however, a marked toxicity is again noted; and the alcohols with nine and ten carbon atoms, respectively, are the most toxic of the whole series studied. Passing to undecyl alcohol, the toxicity begins gradually to decrease again; and the rest of the series, with slight variations in case of pentadecyl and hexadecyl alcohols, are comparatively innocuous. The relative toxicity of the whole series of eighteen primary alcohols studied is strikingly illustrated in the subjoined curve, in which the ordinates express the coefficient of growth while the abscissae indicate the molecular weight of each member.

A number of experiments, performed with mixtures of the various members of the series studied, illustrated the pharmacological phenomena of antagonism and synergism (1929), as may be seen from table 2. It will be noted that combinations of some of the members, as, for instance, ethyl and methyl alcohols, had an antidynamic or antagonistic effect; in other words, a mixture of these two alcohols produced less toxicity than might have been expected from a simple summation of the effects of the two components. A similar antidynamic effect was produced by a combination of ethyl and propyl alcohols. Other mixtures or combinations, on the contrary, indicated an increase in toxicity, as compared with the relative toxicity of the two components, or, to use a pharmacological term, produced a synergistic

Relative Toxicity of Primary Alcohols of the Aliphatic Series for Lupinus Albus
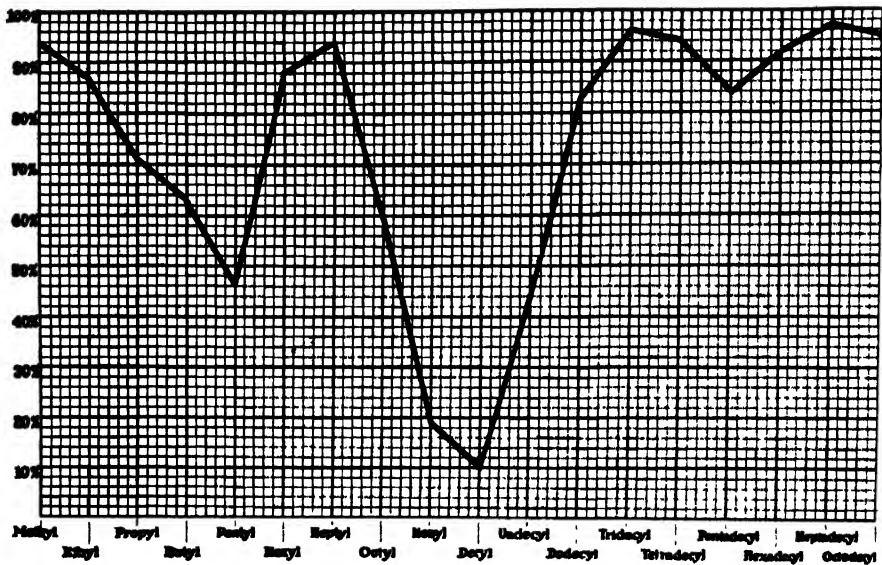
TEXT FIG. 1

effect. Thus, for instance, a combination of ethyl and butyl alcohols, or of ethyl and amyl alcohols, revealed far more toxicity than could be explained by a simple summation of the relative pharmacological effects of the individual components. The two alcohols potentiate the activity of each other. The writers noted similar results in regard to the relative toxicity of the individual alcohols of the aliphatic series as well as of their combinations in experiments on animals. These will be published separately.

SUMMARY

1. The effect of the first eighteen alcohols of the aliphatic series was studied on the growth of *Lupinus albus* seedlings.

2. The results show that the toxicity of the alcohols does not increase

progressively with their molecular weight, contrary to Richardson's law, which was found to hold good for only the first five members of the series.

3. Secondary alcohols, as a rule, are less toxic than primary alcohols for the growth of seedlings.

4. Combinations of various alcohols, in some cases, produce antagonistic and, in other cases, synergistic pharmacological effects.

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THE OVULE AND EMBRYO SAC OF *SAXIFRAGA VIRGINIENSIS*

MARJORIE CHAPMAN

In the course of investigations of the floral anatomy of *Saxifraga virginiensis* it was noticed that, in transverse sections of the flower, the ovules are so oriented that their structure can be well observed, that they are clearly differentiated by the stain, and that the embryo sac structure is especially clearly shown.   Despite the smallness of the ovary it seemed as if, for several reasons, sections of it might profitably be used to illustrate the development of the ovule and of the embryo sac.   The position of the species among the dicotyledons makes it representative of the great majority of the spermatophytes.   Its distribution, from New Brunswick and Quebec south to Georgia and west to Minnesota, Missouri, and Tennessee (6), makes it available to many.   Further, the arrangement of the flowers in the inflorescence is such that it is possible to obtain, in a single cluster, flowers in various stages of development, from very young buds to those in the full bloom of maturity.   For these reasons, then, to which are added the two first mentioned, ease of orientation and clearness of structure, it appeared that it would be worth while to trace the development of the ovule in this plant to discover whether it parallels that considered normal and is therefore suitable for use in illustration of the embryogeny of that structure.

LITERATURE

Although no references could be found in the literature to studies of the species *Saxifraga virginiensis*, other members of the genus have been used to illustrate various points in ovule development.   A summary of the references will indicate the trend in the theories of embryo sac formation. In 1877 Warming (11), in connection with the origin and development of the tissues about the embryo sac, mentions that in *S. crassifolia* the "cellule mère primordiale," a cell apparently corresponding to the one designated today as the archesporial cell, divides with a transverse wall, and that the cell thus formed is still in evidence up to fertilization.

Vesque (10) records that in *S. palmata*, at the time when the integuments begin to form, there is a division in a subepidermal cell to form the initial of the "calotte," a capping group of cells in the micropylar region of the nucellus, and the primordial mother cell.   Vesque continues his observations of the developing ovule: "The mother cell divides to form two cells separated by a cell wall, the superior (the outer) dividing again to form the

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AMERICAN JOURNAL OF BOTANY, VOL. 20, No. 3, MARCH, 1933

151

special mother cells, the middle one of which is very small, especially smaller than the inner or inferior cell." According to his idea of the further development of the ovule the two outer cells unite and increase enormously in size.

In 1882 Guignard (2) opposed the idea of the development of the embryo sac of *Saxifraga* as it had been set forth by Vesque. He agreed with the earlier writer that three daughter cells are formed from the primordial mother cell and that the two superior are smaller than the inferior. He differed, however, in his assertion that it is the inner of the three cells which develops into the embryo sac. He found no further anomaly in the constitution of the embryo sac of *Saxifraga*, noting that with stains the antipodal cells are easily found.

Juel (3) was more interested in the conduct of the nucleus in cell division and in the embryo sac after fertilization than in the earlier stages of its development. He described the appearance of the embryo sac as it is preceding fertilization: "Both synergids are lengthened and have their nuclei in the middle of the cell. The basal parts of the synergids are paralleled by the egg cell, the further end of which pushes forward into the embryo sac cavity and contains the egg nucleus. The central nucleus lies in, or somewhat under, the middle of the embryo sac and is embedded in a much-vacuolated plasma mass. Between it and the egg apparatus lies a great vacuole which is crossed by somewhat thickened plasma strands binding the egg cell to the central nucleus. The antipodals are usually small. Their plasma is thick, without vacuoles."

Pace (5) recorded that, as far as her examination had gone, the megaspores are always in a row and that the fourth forms the embryo sac. According to her observations the synergids have a filiform apparatus and there is a notch marking them off from the rest of the embryo sac. She found the primary endosperm nucleus usually occupying a position near the base of the sac.

Schurhoff in 1925 (9) and Marsden-Jones and Turrill in 1928 (4) were concerned more particularly with the strictly cytological structure of the ovules of various *Saxifraga* species.

Summarizing the work on embryo sac development, Schnarf in (8) indicated that of the six species of the genus *Saxifraga* which had been examined all were of the normal type, one which corresponds to the description of Juel. He mentioned, however, that in *S. granulata*, according to the work of Juel, there may occur the so-called T-arrangement of the megaspores. He, likewise, records the occurrence of a row of three megaspores, a condition ascribed by him to the rapid disintegration of one of the four original megaspores.

MATERIAL AND METHODS

The material which was used in this investigation was collected May 5, 1931, about 2:00 o'clock P.M. The inflorescences were killed entire in a

solution of chromic, acetic and osmic acids, the formula for which was supplied by Dr. L. F. Randolph (7 gm. chromic, 10 cc. acetic, 1982 cc. water; to 20 cc. of which are added two drops of 2 percent osmic acid before using). This killing solution was especially effective since the contents of the cells were well preserved and cell division stages were numerous in sections cut from the material. From the killing solution the flowers, after being thoroughly washed, were run into 70 percent alcohol in which they were kept until they were used in the spring of 1932.

Material was most successfully embedded from butyl alcohol according to the method of Zirkle (12). Transverse sections of the flowers were cut 10 microns in thickness and were found to be satisfactory, showing, when properly oriented, all of the nuclei in one section.

Three different stains were used. For preliminary examination of the material safranin alone was satisfactory provided destaining was carried rather far. A differential staining with safranin and light green showed the structure of the embryo sacs very clearly. For more particular examination of the material, sections stained according to the iron alum-haematoxylin method, with the stain rapidly ripened according to the directions of Camp (1), were found to show most clearly the structure of the parts studied. Division stages were particularly well differentiated by this stain.

RESULTS

The ovary of the genus *Saxifraga* is made up of two carpels united at their bases but separated above and extended to two separate styles. The placentation is axile, each carpel developing at the axis two separate placentae. Numerous anatropous ovules are formed on each of these. A short distance above the point where the carpels separate there are in the outer ovary tissue darkly staining groups of cells, rich in contents, which probably serve as nectaries.

In *S. virginiensis* the earliest stage of ovule development which was observed shows the young ovule as a protuberance from the placenta. The epidermal layer is rounded by anticlinal divisions. Directly beneath that layer lies a large cell, the archesporial cell, with contents denser than those of the surrounding cells. From the archesporial cell will be formed the initials of the sporogenous tissue and of vegetative tissue about it. The divisions of the archesporial cell were not observed.

At the megaspore mother cell stage the ovule has reached greater complexity. The funiculus, which is still short, has projected the structure into the loculus. The ovule is bending over into the anatropous position of its mature state. Small bumps on either side indicate the position of the integument. There are three layers of nucellar cells immediately surrounding the megaspore mother cell. That cell is enlarged and elongated and contains a thick homogeneous cytoplasm. Its outstanding feature is a very large nucleus which usually lies in the micropylar end of the cell.

15

A characteristically large and darkly staining nucleolus surrounded by a relatively clear area is enclosed by the nuclear membrane. Occasionally two nucleoli mark the nucleus of the megaspore mother cell and frequently the irregularly arranged chromosomes of the early prophase of division help to obscure the disappearing nucleolus with their intermingled threads.

The prophase of the division of the megaspore mother cell was found frequently on the slides which were examined. Less frequently other stages in that first division were seen. Several metaphases (pl. 4, fig. 1) indicate that the division proceeds so that the first wall dividing the mother cell is formed transverse to the longitudinal axis of that cell. The fact that very few stages that could definitely be called two-celled were observed would seem to indicate that the second division in the formation of the megaspores takes place fairly soon after the first has been completed.

It is in the orientation of the two spindles of the second division and in the consequent arrangement of the four megaspores that *Saxifraga virginiensis* deviates from the normal in its embryo sac formation. Of the two cells formed by the first division the chalazal is usually the first to proceed in the second division. In that cell the spindle is formed parallel to the longitudinal axis of the ovary in a position which is quite normal. The spindle of the micropylar cell, however, lagging a bit behind the other in its appearance, forms parallel to the transverse axis (fig. 2). Such a division results in the so-called T-shaped arrangement of the megaspores, the occurrence of which is discussed by Rutgers (7) and summarized by Schnarf (8). Juel's remark (3) that "the end wall between both uppermost cells is usually oblique" and the drawing with which he illustrates that condition would appear to show a similar arrangement of the megaspores in the related *S. granulata*.

The four megaspores in the T-shaped form were not frequently seen (fig. 3). The scarcity of sections of that stage is probably explained by the rapid disintegration of the three non-functioning megaspores. While the cell in the chalazal end is increasing in length, the three outer cells are being compressed and are disintegrating and usually appear as two dark streaks of structureless material bunched below the functioning megaspore. The two cells lying side by side early lose their distinctness, and through all of the stages following, the appearance of the disorganized mass of megaspore tissue, frequently separated transversely, might easily lead to the conclusion that there were but three megaspores. At this stage the megaspore contains a large nucleus, frequently with two large nucleoli, surrounded by rather homogeneous cytoplasm. From the time of maturity of the megaspore mother cell through that of the megaspore formation the surrounding tissues do not change markedly in appearance. The integument tissue extends a bit farther down around the nucellus.

At the binucleate stage of the embryo sac (fig. 4) several changes are noticeable in the ovule. The embryo sac has increased in length and the

appearance of an individual structure embedded in a mass of different tissue is more evident. The cytoplasm of the sac is marked by small vacuoles which are concentrated in the region between the two nuclei which lie at either end in a somewhat denser cytoplasm. The chalazal nucleus may be separated from its end of the sac by cytoplasm almost as greatly vacuolated as that between the two nuclei. The nuclei are outstanding in size and in the prominence of their nucleoli. The three disintegrating megaspores appear as a crowded and crushed mass beyond the embryo sac. At about this time the tissue about the sac begins to assume greater distinction due to the denser contents of the three or four layers of nucellar cells. The integument tissue has become extended to the end of the other tissues and the whole ovule is turned in a position nearly anatropous.

The formation of the four nuclei from the two is accomplished by divisions which are not remarkable except in the rather frequent arrangement of the two spindles almost at right angles to each other. The spindle at the micropylar end of the embryo sac is usually formed at right angles to the longitudinal axis of the sac, whereas that of the division of the nucleus at the other end usually parallels the axis. In consequence of this orientation, the four nuclei are usually arranged with the two at the micropylar end lying, apparently, side by side, whereas those at the chalazal end are placed one above the other. The growth of the embryo sac proceeds fairly rapidly and is accompanied by an increase in the size of the vacuoles. Many of those between the nuclei coalesce so that the center of the embryo sac is almost entirely vacuolated. The tissue of the integument has projected beyond the rest of the ovule and begins to surround it almost completely.

The division of the four nuclei which results in the eight nuclei normal in a mature embryo sac is not marked by anything unusual. Neither a sufficient number of these divisions nor of the nuclei immediately after the formation were observed to determine whether there is any regularity in their arrangement. The eight nuclei, four in each end of the embryo sac, are at first very similar in their appearance. The two groups of four are separated by the vacuole, which has been previously mentioned, in an embryo sac which has become further lengthened.

The similarity of the nuclei is a characteristic not long retained. Very shortly three of the nuclei of the chalazal end of the sac become marked as the antipodals because of their smallness and because of their crowded position in that end (fig. 5). A constriction between the cytoplasm immediately about the antipodals and that of the main body of the embryo sac frequently makes these nuclei appear detached. In the later development of the antipodals they seem to be surrounded by membranes, which are formed last in the regions adjoining the main body of the embryo sac. The antipodal cells do not persist for any extended length of time. They are infrequently seen when the embryo sac reaches the stage before fertiliza-

tion when the polar nuclei are fused and the egg apparatus fills the micropylar end of the sac. It is interesting in this connection that Vesque (10) indicated that the *Saxifraga* embryo sac is made up of one "tetrad" having in the mature stage a sexual apparatus and a central nucleus. Due to the brief existence of the antipodals and to cruder methods of preparation either he failed to see antipodal cells or he classed them as "anticlines," a mistake which Guignard (2) corrected after his observations of the antipodal regions of several members of the genus.

The fourth of the original group of four nuclei in the chalazal end migrates through the vacuolated center of the embryo sac until it comes in contact with a nucleus which has moved up from the micropylar region (fig. 5). These two polar nuclei unite and the one large nucleus formed by their union maintains a position in the middle, or somewhat above the middle, of the embryo sac until fertilization. This large fusion nucleus is the most striking structure of the older embryo sac (fig. 6). It has a large prominent nucleolus which is surrounded by an area fairly free of granular content. Close to the nuclear membrane are scattered granules among which there are sometimes some which are larger than those commonly present.

The nuclei of the egg apparatus are at first distinguished by growth in size (fig. 5), later by their position. The two synergids, situated more closely in the micropylar end of the embryo sac, are each apparently surrounded by a membrane which encloses a pear-shaped mass of cytoplasm. The egg cell is usually placed somewhat higher in the position characteristic in the normal embryo sac. The nuclei of the synergids are usually situated a little above the center of the cell and are surmounted by a vacuolated region. The filiform apparatus described by Pace (5), as usual in the species of *Saxifraga* at which she had looked, is not a marked feature of *S. virginiensis;* in fact, there was nothing observed which was sufficiently definite to be designated "filiform apparatus." A separation of the pointed ends of the two synergids was rather frequently noticed (fig. 6).

The usual position of the egg cell in the older embryo sac is one which is normal in a mature sac—toward the center from the synergids (fig. 6). The egg cell is sometimes, however, more closely on a line with the synergids than beyond them. It is separated from the other structures by a rather indefinite membrane which appears as a somewhat more dense line of cytoplasm. The nucleus is usually distinguished by its size from those of the synergids. This distinction is not always very marked. It is necessary to combine a comparison of position and size in order to separate the egg nucleus from those of the synergids.

The tissues about the mature embryo sac exhibit some interesting features. The single integument completely envelops the structures within it except for a central opening, the micropyle, which will permit the passage of the pollen tube. The outer boundary of the nucellus is marked by a

row of cells larger than and different in their staining properties from those nearer the embryo sac. The two layers of the nucellus which border the micropylar and lateral regions of the embryo sac, although they stain more darkly and have more contents than the cells of the integument, are not as darkly stained nor as full of contents as the cells which cap the chalazal end. In that region the cells are smaller, more numerous, and less regularly arranged.

The occurrence of two embryo sacs in the same ovule is not rare in *S. virginiensis*. In sixty-seven flowers sectioned that condition was observed in five different cases. All of the extra embryo sacs were developed from a megaspore resulting from the division of a second megaspore mother cell. They were not formed by the development of sister megaspores. In three of these cases each of the embryo sacs was surrounded by its own nucellus. Between the two, where the nucellar tissue of one was in contact with that of the other, the cells were crowded and pushed together. These three more mature pairs were in stages of two and four nuclei. Two of the pairs of embryo sacs were in much earlier stages. In one, the megaspore mother cells were in prophase of division, whereas in the other they were in the metaphase of that first division. In one of the last two examples the megaspore mother cells lay side by side unseparated by other tissue. In the other they were separated by two or three layers of the nucellus.

SUMMARY

1. The four megaspores of *Saxifraga virginiensis* are formed in a T-shaped arrangement. It is the inner of the four megaspores which develops into the embryo sac.

2. The eight nuclei of the embryo sac are formed in the normal way.

3. The antipodal cells are small and disintegrate early.

4. The polar nuclei fuse some time before fertilization to form an outstandingly large fusion nucleus.

5. The embryo sac, as it usually appears just before fertilization, is seen with four nuclei—the two synergids and the egg arranged, as normally, in the micropylar end of the embryo sac and the fusion nucleus in about the middle, or somewhat above the middle, of the structure. Antipodal cells may be in evidence at this stage although they are usually too much disintegrated to be outstanding.

6. Two embryo sacs in one ovule were observed in several of the ovaries sectioned.

7. Although the structure of the embryo sac is clearly shown in sections of the ovule of *S. virginiensis*, that plant is not remarkably good for illustration of the embryo sac development, because in the stage before fertilization, when the other nuclei are mature, the antipodal cells are infrequently seen.

The writer wishes to acknowledge her indebtedness to Professor Arthur J. Eames, by whom this problem was suggested and under whose direction the work has been completed.

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EXPLANATION OF PLATE 4

Fig. 1. Megaspore mother cell in metaphase of division.

Fig. 2. Two daughter cells of megaspore mother cell in metaphase of division showing the arrangement of the spindles.

Fig. 3. Four megaspores in T-shaped arrangement.

Fig. 4. Binucleate stage of embryo sac with the three non-functioning megaspores below. Distinction between the two outer cells not evident.

Fig. 5. Eight nuclei with those different in function to be distinguished by different positions and differences in size. Two polar nuclei migrating for fusion.

Fig. 6. Four nuclei of embryo sac immediately preceding fertilization. Large fusion nucleus and egg apparatus evident.
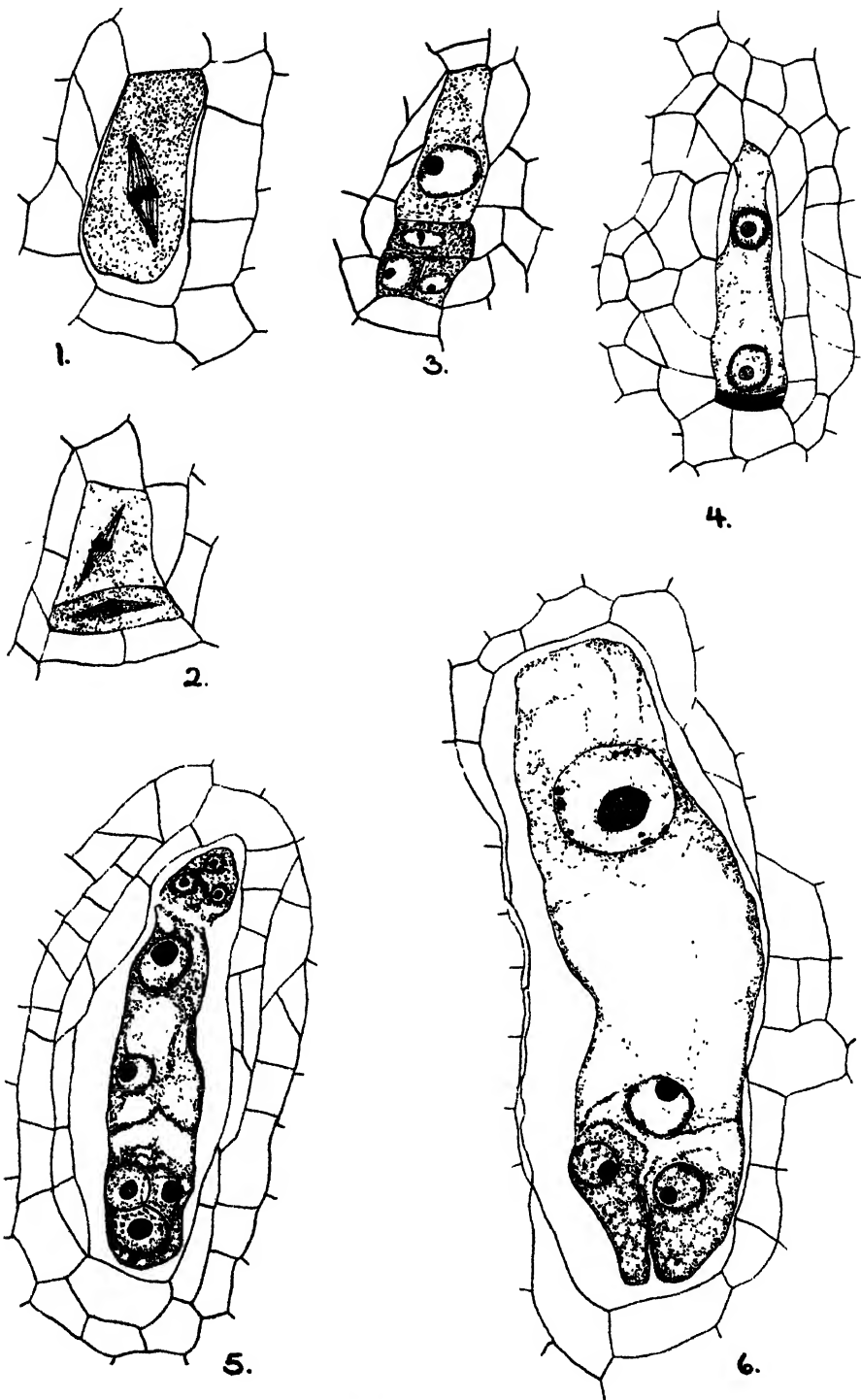
INTUMESCENCES ON POPLAR LEAVES. II. PHYSIOLOGICAL CONSIDERATIONS [1]

CARL D. LA RUE

INTRODUCTION

In preceding papers (12, 13) the writer has described the occurrence and structure of intumescences on leaves of *Populus grandidentata, Populus tremuloides, Eucalyptus coccifera, Eucalyptus cornuta, Hieracium venosum, Mitchella repens,* and *Thurberia thespesioides.* In the present paper an account is given of an attempt to determine the factors involved in stimulating the cells to the unusual growth, which result in these abnormal protuberances.

The hypotheses which have been developed to account for the induction of intumescences have been almost as numerous as the records of their occurrence. Sorauer (18) believed that metabolic disturbance due to an excessive water supply and low assimilation accounted for the outgrowths. In a later paper (19) he added another factor to the complex, namely, the effect of a high temperature.

An excessive water supply due to low transpiration and a high rate of water absorption by the roots, was advanced by Atkinson (1, 2) as the stimulus to formation of oedemas on the tomato and the apple, though he considered that low illumination was favorable also, since it led to the development of "weak, watery tissue."

Steiner (20) discovered intumescences on leaves of *Ruellia formosa* and *Aphelandra Porteana* which he believed were caused by high humidity. By subjecting leaves of *Aphelandra* to a saturated atmosphere he was able to induce these outgrowths, but he failed to secure them by treating the leaves with copper sulfate, ammonium chlorid, formic acid and various other chemicals; by keeping them in the dark; or by submerging them in water.

Douglas (5) induced intumescences on leaves and stems of potato plants by covering them with bell jars. Since the intumescences occurred in either strong or weak light, but not in total darkness, she concluded that light as well as humidity played a part in the abnormal stimulation of the plants. She found also that the abnormalities could be produced by treating young potato plants with copper compounds, as Von Schrenk (23) had previously found in the case of cauliflower.

Küster (11) secured intumescences on leaves of *Populus* floated on water in the presence or the absence of light. In strong light, however, the

growths were best developed on the side of the leaf in contact with the water and this, he believed, was due to the retarded transpiration on that side.

Dale (3) found that high humidity was necessary for the development of intumescences on *Hibiscus vitifolius*, but that light conditions were important also, since the developments occurred in white, red, or yellow light but not in blue, green, or weak light, or in darkness.

Intumescences on leaves of grapes grown in the greenhouse were believed by Viala and Pacottet (22) to be due to intense light and high humidity.

Haberlandt (6) painted thalli of *Conocephalus conicus* and *C. suaveolens* with a one percent solution of mercuric chlorid in alcohol, which killed the cells of the hydathodes. The development of intumescences, which he regarded as "substitute" hydathodes, resulted; due, he believed, to excess pressure developed in the absence of the normal hydathodes.

Smith (17) found that ammonia and Carnoy's fixing fluid gave intumescences on the leaves of cauliflower. He was able to induce crown-gall formation in stems of *Ricinus communis* by injecting by-products of *Bacterium tumefaciens*.

Harvey and Rose (8) and Doubt (4) found that ethylene causes abnormal growths in the tissues of various plants. Wallace (24) found that ethylene caused abnormal growths in sixteen different species of woody plants including the apple. Later (25, 26) he made an intensive study of the intumescences on *Pyrus malus* var. Transparent, induced by treatment with ethylene.

Wisniewski (27) found that intumescences in the lenticels of *Ficus australis* and *Ficus elastica* could be induced by coating the twigs with paraffin oil. He regarded the reduction of transpiration as a major factor but thought that other conditions, not yet fully understood, had an influence in the process.

Schilling (15) reported that coating stems of *Aesculus hippocastanum*, *Spiraea concinna*, *Philodendron pinnatifidum*, *Artocarpus incisa*, and *Sambucus nigra* with vaseline or paraffin oil caused outgrowths, similar to those described by Wisniewski, from the lenticels. He believed that the developments were not due to the chemical stimulus of the vaseline, or paraffin, but mainly to the reduction of transpiration, and possibly to oxygen deficit.

Hahn, Hartley, and Rhoads (7) found that intumescences were produced on the roots of several species of conifers by an excess of soil water, though they did not find that lowered transpiration was a significant factor.

Wolf (28) attributed the intumescences which he observed on leaves of cabbage to injury by wind-blown sand. On the same plant R. B. Harvey (9) induced intumescences by exposure to low temperatures.

In his first paper on this subject (12) the author has told of the production of intumescences on leaves of *Populus grandidentata* and *P. tremuloides* which had been enclosed in a damp chamber; and under any conditions,

such as rolling the leaves, fastening two leaves together, etc., which simulated such an enclosure. Obviously, under such conditions reduced transpiration of the leaves and high humidity of the atmosphere would result. Such conditions are usually conducive to growth and it was not illogical to suspect that water played a major part in the growth of the intumescences.

THE ROLE OF WATER

Intumescences on poplar leaves have been found in nature by the author only on leaves rolled up by leaf-rolling insects and on those stuck closely together by web-spinning insects. These were found only after the overgrowths had been induced in damp chambers, and the similarity of conditions indicated great likelihood that there was an identity of causal factors.

An extensive series of experiments showed that any condition which led to the leaves being surrounded by an unchanging atmosphere and an ample water supply would induce the formation of intumescences. Under the following conditions these growths were almost invariably formed: (1) enclosure in damp chambers and under bell jars, of leafy twigs with cut ends in water; (2) rolling of leaves by insects, or experimentally; (3) sticking leaves closely together by insects, or experimentally; (4) floating leaves on water in enclosed chambers, with either the upper or the lower surface in contact with the water; (5) floating leaves as in the preceding, but on culture solutions such as Knop's, Pfeffer's, Robbins's, or Benecke's; (6) placing segments of leaves on 1½ and 2 percent agar media in Petri dishes, the agar media being made with water only, or with Pfeffer's, Robbins's, or Benecke's nutrient solution; (7) enclosure of leafy twigs on trees, in flasks or bottles. Enclosure of single leaves on trees in test tubes. Covering of surfaces of leaves with vaselined or varnished paper so as to form a chamber between leaf and paper; (8) covering a portion of leaf surface with a chamber constructed of cork.

The effect of excessive water supply on leaves in the open air

All the foregoing tests pointed to the need of water in the development of the abnormalities. Whether or not water alone could induce their development was first tested by subjecting leaves to a continuous spray. As no convenient nozzle for producing a fine spray was at hand, one was made by wedging a short segment of grape-vine stem in a small water tap. As the water was forced through the vessels of the stem it was broken into a fine mist which served the purpose. Leafy twigs were cut, set in jars of water, and placed under the spray, but otherwise exposed to the open air. Here they were kept dripping with moisture four or sometimes five days. Although large numbers of leaves were used in the experiments, and special care was taken in the examination of those which had been constantly wet for the whole period, not a single intumescence was found. Control twigs from the same branches which had been put into water and enclosed in damp chambers showed innumerable intumescences on their leaves.

To determine whether an adequate internal supply of water was essential for the production of overgrowths—a condition which may not have been satisfied in the preceding experiment—the cut ends of leafy twigs were pushed through rubber stoppers which were secured in water taps, after which they were subjected to the full pressure of the water system. Water was injected into the twigs with such force as to cause it to drip from cut petioles. In two hours the leaves became suffused with water and dark in color, but water did not exude from them except where they bore recent cuts or tears. Old cuts and insect injuries had become so well cicatrized as to hold back the water as well as the normal epidermis did.

The leaves on these twigs were left in the open air, and on dry days they would show by their changed appearance that they had become dried to a normal water content. But on humid days they became dark and injected with water again, while on rainy days, drops of water, apparently condensed from the atmosphere, appeared on their surfaces. Though the conditions may appear to have been abnormal, the leaves showed no ill effect, but remained normal, save for the intermittent appearance of being suffused with water, for a remarkable length of time. At the end of two weeks no leaves had died, yellowed, or been dropped, though petioles from which leaves had been cut had abscissed in the meantime.

No accurate records were made of the relative lengths of time during which the leaves appeared injected or normal, but there is no doubt that the injected condition predominated. During this period leaves on twigs merely cut and placed in water in the open air dried out and withered in a few hours. None of the leaves of the experiment withered or showed any wilting at any time, and there can be no question that an adequate if not an excessive water supply was present at all times, but no intumescences ever developed. Control leaves in damp chambers showed the usual crop of abnormal growths.

A set of twigs was arranged in water taps as in the preceding experiment, but the leaves instead of being allowed to become dry externally were placed under a continuous fine spray of water. Here the maximum supply of water possible to leaves in an unenclosed atmosphere was given. That it was greater than that ordinarily available to leaves in damp chambers, either where cut twigs were used or twigs still attached to the tree, is shown by the fact that under the latter conditions the leaves never became injected with water as these did. But even with this abundant water supply no intumescences developed.

Behavior of submerged leaves

Experiments with submerged leaves, and observations on many other experiments in which leaves became submerged accidentally, have shown that these leaves never develop intumescences. Leaves partially immersed usually show a very fine development of outgrowths on the unsubmerged parts.

Although the foregoing experiments show conclusively that the presence of an excess of water, either in the atmosphere or in the tissues of the plant, is of itself insufficient to induce the formation of intumescences, further experiments were made to determine what part water does have in the process.

Behavior of leaves in damp chambers without external water supply

An experiment was made with leaves on twigs inclosed in a damp chamber, but without any water being supplied to the cut ends of the twigs. Here the leaves were surrounded with an atmosphere of high humidity, but only the water already in them and in the twigs when they were cut was available. Since transpiration was greatly reduced, the leaves did not wilt, but only a few small intumescences were developed. Control leaves on twigs set in water and enclosed in a damp chamber developed large and very numerous intumescences.

The results of surrounding leaves with dry air in closed chambers

Two experiments were run with leafy twigs, the ends of which were placed in a jar of water which was sealed to prevent evaporation from the water surface. The base of the jar was set in a dish of fused $CaCl_2$ and the whole assembly was then covered by a bell jar. In these tests the outer leaves bore no intumescences though they were not abnormally dry, but the inner leaves, which were somewhat massed together, produced very numerous ones. The crowded leaves were wet with water condensed from transpired water vapor, and from the center of the mass toward the outside the leaves became increasingly dry. The number of intumescences decreased toward the outside with the decrease in moisture.

Other experiments were made with the leaves well separated and with an abundant supply of $CaCl_2$ in the chamber. Intumescences did not develop on these leaves, which usually dried and withered in a few days.

To make sure that the leaves had an adequate amount of water given them through the stems, cut twigs were set in rubber tubes into which water was run from a tank eight feet above. The leaves were then enclosed in a chamber with $CaCl_2$, where they dried out completely in a few days without producing any intumescences.

Another set of twigs was treated as in the preceding test except that water was injected into them from a water tap. The leaves on these twigs behaved exactly as did those with water forced into them under a lower pressure.

It appears that leaves enclosed in a stagnant atmosphere will not develop intumescences unless the humidity of the atmosphere is high, no matter how much water may be supplied internally from the twig.

The results of forcing water into leaves enclosed in damp chambers

It has been shown by experiment that if the twigs are not supplied with water, the intumescences developed on the leaves are few and small. To discover whether or not an excess of water supplied through the twigs would increase the number, or the size, or the rate of development of the outgrowths, leafy twigs were set in tubes with water forced into them from a tank eight feet overhead, and the leaves were enclosed in a damp chamber. It could not be seen that the intumescences were in any way different from those on control leaves in damp chambers on twigs merely placed in water.

A repetition of the test with an increased pressure in tubes attached to a water tap gave the same result—from which it appears that twigs with their cut ends in water are able to supply a sufficient amount of water for the development of intumescences on leaves in damp chambers. An excess of internal water is not the cause of intumescences; nor is it even an aid to their development.

Since an excess of water supplied to the leaves, internally and externally at the same time, will not initiate intumescences, it is apparent that water plays a minor rather than a major role in their causation.

THE EFFECT OF LIGHT

Light has been considered an important factor in the growth of intumescences by a number of investigators. Some have found that low light intensity, resulting in a low rate of assimilation, is conductive to the abnormal growths; while others have discovered that full illumination was required for their development.

Most of the experiments described in this paper were performed in a laboratory in light from a north window, so that it was known, from the first discovery of the existence of the intumescences, that they would develop well in weak light. A number of experiments were made to test the effect of various light intensities, and from them, and from other experiments made primarily to study the effect of other factors, it was found that the growths appeared on leaves of *Populus grandidentata* and *P. tremuloides*, under all the following conditions: (1) in weak light in laboratory; (2) in darkness in laboratory; (3) in shade, twigs attached to tree; (4) in sunlight, twigs attached to tree; (5) in shade, leaves on tree, but rolled up by insects, or experimentally; (6) in sunlight, leaves on tree, but rolled up by insects, or experimentally; (7) in shade, leaves on tree, but stuck together by insects, or experimentally; (8) in sunlight, leaves on tree, but stuck together by insects, or experimentally; (9) in weak light, upper surface of leaves shaded, experimentally; (10) in sunlight, upper surface of leaves shaded; (11) in shade, paper fastened on surface; (12) in sunlight, paper fastened on surface.

Under all these conditions, which represent a great variety of light intensities ranging from full insolation to complete darkness, the intumes-

cences were unvarying. No differences could be detected in their abundance, their structure, or the rate of their development which indicated that one light condition was more favorable to their initiation than another.

Inasmuch as the leaves in all cases seem to contain sufficient food to develop intumescences, even the indirect effect of light as a producer of food seemed to be of no consequence.

FOOD SUPPLY AND MINERAL NUTRIENTS

Early in the study of this problem it was demonstrated repeatedly that intumescences developed on detached leaves floated in water, either in light or darkness. Apparently the leaves did not need the extra food which might have resulted from photosynthesis in their tissues. Halves of leaves, even, contained sufficient food for a full development of intumescences.

However, it cannot be said that the relative abundance of food is of no importance in these growths, for if leaves attached to twigs are floated on water, the outgrowths are larger and more abundant than those on detached leaves. The twigs to which these leaves were attached were not examined to determine their starch content, but each was about four inches long and could have held an abundant supply of food for much more growth than is represented by a set of intumescences on the leaves. That the twigs contain an ample food supply is shown by the fact that experiments in which leaves attached to the tree were placed in closed compartments did not result in larger or more numerous intumescences than were developed on leaves attached to the four-inch twigs.

It was found that intumescences developed on leaves floated in Pfeffer's solution, Benecke's solution, and Robbins's solution. They developed also on nutrient agar made up with these solutions, but in no case did it appear likely that any substance in these nutrient solutions supplied any need not met by the store of materials in the twig or even in the leaf itself.

The effect of sugar solutions on the growth of intumescences

At the same time as the preceding tests were made, another set of leaves was placed in Pfeffer's solution with the addition of 4 percent dextrose, and on agar made up with this solution. Other leaves were floated in Robbins's solution with 2 percent dextrose added, and others were placed on agar made with that solution. Without exception, the nutrient solutions, and the nutrient agars which contained dextrose, brought about a more rapid and a more extensive development of outgrowths than was found on the corresponding solutions without sugar. Quantitative results could not be secured easily, but it was evident that the development on nutrient agar containing dextrose was superior even to that found on leaves still attached to the tree. This is not to be attributed to a lack of food in the tree, but rather to the fact that the leaves left attached to the tree, but enclosed in a damp chamber, begin and even complete the formation of an abscission

layer before the intumescences have reached their fullest extension.   After abscission, which seems to be stimulated by the enclosure of the leaves in the damp chamber, the leaves contain too small a food supply to compete with richly fed leaf segments on a dextrose agar.   It should be noted that the nutrient agar used was kept sterile, and the leaf segments were sterilized with "Zonite," so that fermentation of the agar was prevented on a number of the plates, though some developed colonies of yeast, and mold.

THE PARTS PLAYED BY CARBON DIOXID AND OXYGEN

By the time the discovery was made that neither an excess of water nor any special condition as regards food supply could account for the initiation of intumescences, it became rather more apparent that the inclosure in a chamber was responsible in some way for the phenomenon. The respiration of the enclosed leaves must have had the effect of increasing the concentration of CO_2, and of lowering the oxygen tension; conceivably, either of these changes might result in the formation of the outgrowths.

It so happens that conditions which surround the leaf with an atmosphere saturated with water vapor also are usually conditions which lead to a stagnation of the atmosphere with the consequences just noted.   Leaves rolled up by insects or stuck together by web-spinning insects are the only ones which have developed intumescences in the woods under natural conditions.   Under these circumstances, closed or partially closed chambers are formed.   Under the discussion of the role of water the rather large number of ways in which leaves were inclosed experimentally have been described.   All of these serve equally well in securing an unventilated atmosphere about the leaves with subsequent decrease of oxygen and increase of carbon dioxid.

The behavior of leaves in ventilated damp chambers

An experiment was devised to allow of change of air, and at the same time to keep a water-saturated atmosphere about the leaves.   Air, charged with water-vapor by bubbling it through four wash bottles containing water, was drawn by an aspirator through the closed chamber containing the leaves.   After the experiment had been run for four days with leaves of *P. grandidentata* and *P. tremuloides*, long enough to produce intumescences under ordinary conditions, only four intumescences could be found on a total of 33 leaves subjected to the experiment.   The control leaves in a chamber identical to that used in the experiment, but unventilated, bore hundreds of intumescences.

A repetition of the experiment gave only three intumescences on 33 leaves, and hundreds on the control leaves.   A third test on 25 leaves showed that three had small scattered intumescences, and two, which had lain close against the wall of the chamber, had an abundant crop of the growths.   Another trial, in which the leaves were crowded closely together,

so that the air may not have passed freely among them, showed numerous outgrowths on a few leaves and none on the others. Two other tests, in which care was taken to prevent leaves from being pressed closely together, did not produce a single intumescence. In all these experiments care was taken to see that the atmosphere about the leaves was thoroughly saturated with water. The control leaves almost without exception produced great numbers of outgrowths. From these results, and those on the effect of water above, the conclusion must be drawn that the stagnation of the atmosphere is more important in the production of intumescences than high humidity can be.

A further confirmation came from trials in which a rather small number of leaves were placed in a large chamber of which the atmosphere was saturated with water. These trials always resulted in the development of a few intumescences on the leaves just above the water in the bottom of the chamber, and none in the upper part of the chamber where the CO_2 content was probably less.

Results with leaves in air of low oxygen content and in rarefied air

A test was made of the effect of lowering the oxygen tension by placing a tube of alkaline pyrogallol in a chamber tightly closed, and with a water-saturated atmosphere. No intumescences developed in this test, or in a repetition of it. Most of the leaves were dead after 10 days but some remained normal.

Another set of leaves was put in a chamber from which the air was pumped out by a rubber bulb operated by hand, and then forced through a series of wash bottles filled with pyrogallol before being returned to the chamber. This procedure resulted in injury, due to oxygen starvation apparently, for in three days most of the leaves were dying. No intumescences had even been initiated except on four leaves which lay against the side of the chamber, where they may have held a certain amount of oxygen inclosed.

The concentration of the air was decreased in two experiments by enclosing leaves in a rubber-stoppered glass jar of one-gallon capacity from which air was exhausted by an aspirator on a water tap in the laboratory. Though the leaves were alive after 10 days under such conditions, no sign of any abnormal growths could be found on either of the species, *P. grandidentata* or *P. tremuloides*. Control leaves, in ordinary damp chambers, showed the usual abundance of intumescences.

Further evidence was gained by placing a set of leaves in a closed jar in which another set of leaves had been enclosed previously for three days. The first set of leaves had produced abundant intumescences, but the second group put into the stale air did not develop a single intumescence. This test was made on leaves of *P. grandidentata*, but a similar one made on *P. tremuloides* developed intumescences very abundantly in four days, a rather

short time for their full development in that species. A study of the situation to explain this anomalous result showed that in this instance only a small number of leaves had been confined in the chamber before the second lot of leaves was put in.

The water in the bottoms of the jars used in the two preceding experiments became rather foul before the second set of leaves was added. To remove the effect of foul, and possibly toxic, water the experiment was repeated, but the second set of twigs was placed in a bottle of fresh water which was then set in the bottom of the chamber. Leaves of *P. grandidentata* were used in this test and the same as in the one where the twigs were in the polluted water.

It seems that in the experiments where leaves of *P. grandidentata* were used, the first set of leaves was a rather large one, and it used up the oxygen to such an extent as to leave the atmosphere too poor to allow any growth of the second set of leaves. The experiment with leaves of *P. tremuloides* was made with only a small number in the first set, which did not lower the oxygen tension too much, but just sufficiently to initiate a rapid development of intumescences on the second set.

There is good evidence from these experiments that oxygen plays an important part in the growth of intumescences, and that when the oxygen tension is too much lowered no outgrowths are formed, even if the leaves are surrounded by an atmosphere charged with CO_2 and water vapor. It is rather to be expected that any great reduction of the oxygen would not leave a sufficient supply to allow of any growth whatsoever. For this reason, apparently, submerged leaves are unable to develop intumescences though they may remain alive for many days. Of course, they may be prevented by their position from receiving the stimulus which initiates outgrowths on the unsubmerged leaves in the same container. On the other hand, the disturbance of respiration due to an excess of CO_2, or a lack of O_2, might be the stimulus to development of outgrowths. So far as the evidence gained by the preceding experiments is concerned, the one might serve as well as the other. Accordingly some trials were made to determine, if possible, the part which CO_2 might play in the process.

Results with leaves kept in an atmosphere free of carbon dioxid

Several experiments were made in which leaves of *Populus grandidentata* were enclosed in a water-saturated chamber in which NaOH or KOH was exposed to absorb the CO_2. None of the leaves developed intumescences, but they soon became pale and unhealthy in appearance and the lack of outgrowths might as readily be attributed to an abnormal condition of the leaves as to the lack of CO_2 in the chambers.

A trial was made with soda-lime to absorb the CO_2. The leaves on five twigs of *P. grandidentata* had no outgrowths, but those on two twigs produced numerous intumescences. The leaves on these two twigs may have

been crowded together so that the CO_2 was trapped between them, so another test was made with five bell jars set over vessels containing soda-lime. Leafy twigs of *P. grandidentata* were introduced into the jars. The leaves in two of these jars became very dry, but those in the other three jars developed intumescences.

To be sure this result was not due to the failure of the CO_2 to reach the soda-lime, another set of leaves of *P. grandidentata* was enclosed in a water-saturated chamber from which the air was drawn, pumped through a train of bottles containing soda-lime, then through wash bottles containing water, and finally returned to the chamber. The air was pumped by a rubber bulb at intervals of about two hours during the day. After 30 hours under these conditions the leaves became very unhealthy in appearance and were covered with black spots which exuded drops of water. The control leaves were green and covered with incipient intumescences. Just why the soda-lime or the NaOH and KOH should have injured the leaves is not clear, but it was evident that these substances were injurious to such a degree as to render them unfit for further use in experiments.

Barium hydroxid was tried next as a means of removing CO_2 from the chamber, and an experiment was made exactly like the preceding one except that a solution of barium hydroxid was substituted for the injurious soda-lime. The leaves remained healthy and all developed intumescences. A repetition of the experiment gave the same result. The possibility that the accumulation of CO_2 during the seven hours of the night, when the air was not circulated, might have initiated the development of the abnormal growths still existed.

Since the barium hydroxid did not harm the leaves, an experiment was set up with twigs of *P. grandidentata* in a jar of water which was set inside a larger jar filled nearly to the top of the inner jar with barium hydroxid. This brought the leaves very near the surface of the barium hydroxid solution. The top of the jar was covered with a glass plate sealed in place with vaseline. Under these conditions a heavy scum of barium carbonate soon formed on the surface of the hydroxid solution. In four days nearly all the leaves were covered by intumescences; even those which dipped in the solution had growths on the unsubmerged parts.

To secure a certain circulation of air about the leaves a set was placed in a chamber from which the air was drawn out and passed through a series of bottles containing barium hydroxid before it was returned to the chamber. But in this experiment, unlike some of the preceding ones, the air was pumped by a device developed by Osterhout (14), which kept the air in constant circulation and carried the CO_2 out of the enclosed chamber as fast as it was formed. As in most of these experiments, *P. grandidentata* leaves were used. Control leaves were enclosed in a chamber without circulation of air. Many leaves in the experiment, and also many among the control leaves, had no outgrowths, but there were a number under each of the conditions

which bore a considerable number of intumescences. Quite as many intumescences were developed in the experimental lot as in the controls. Since the leaves were rather crowded in the experimental chamber, it was impossible to be sure that the intumescences had not been developed on leaves pressed against the walls of the chamber, or against each other, so that the CO_2 was trapped around them. A new experiment was planned to obviate this defect.

The problem of enclosing a number of leaves on twigs set in water, in a chamber in such a way as to keep each leaf in full contact with the air and entirely separated from the others and from the walls of the chamber, required the use of a special device. Finally the idea of using the metal-wire screening known as "hardware cloth" suggested itself. Two cylinders were made of this material, one of which fitted inside the other with sufficient space between to keep the leaves attached to them from touching at any point. Twigs of varying lengths were selected so that the leaves on them could be spread over the cylinders, while the bases of the twigs were submerged in water. The leaves were spread over the metal fabric and fastened in place with segments of a small tinned wire so that none of the leaves overlapped but nearly all the surface of each of the cylinders was covered. The larger cylinder was then placed around the smaller one and both were set on a glass plate and covered with a tall bell jar lined with saturated filter paper. Water was then poured into the bottom of the chamber thus formed so that the ends of all the twigs were in water, and the filter-paper lining of the chamber was kept wet. The leaves were now entirely exposed to the air except for the very small amount of surface in contact with the wires, beneath which very little CO_2 could accumulate. An inlet tube into the chamber was fitted with four outlets above the leaves to distribute the incoming air. The exhaust tube was lowered into the chamber so that its opening rested just above the water level, and CO_2 could not accumulate in the bottom of the chamber. The air was pumped continuously from the chamber, bubbled through four bottles containing barium hydroxid solution, and through two bottles of water, and returned to the chamber. Osterhout's device was used to secure a constant flow of air during the whole duration of the experiment.

A set of control leaves was prepared in exactly the same way except that the barium hydroxid solution was omitted, and the circulating air was passed only through bottles of water.

At the end of five days all the leaves were examined, and very numerous and very well-developed intumescences were found on nearly every leaf of the experimental set, and of the control group as well.

A repetition of this experiment gave the same result. In both these tests no difference could be seen between the experimental and the control leaves. No CO_2 could have accumulated in the experimental chamber, as the barium hydroxid solution was changed at intervals and the last of the

train of bottles of this solution never showed any precipitate of barium carbonate.   No CO_2 was removed from the control chamber except such as was absorbed in the water in the wash bottles.   The evidence is conclusive that the accumulation of carbon dioxid around the leaves plays no part in providing a stimulus for the development of intumescences.

THE EFFECT OF VOLATILE SUBSTANCES GIVEN OFF BY THE LEAVES

It was noticed in the first tests in which considerable numbers of leaves were kept in a closed chamber that a strong odor could be detected in all the chambers after the leaves had been confined therein for a few days. The odors differed slightly with different species, so that one could distinguish readily the chambers in which a given species had been kept.   *P. deltoides* always produced a stronger odor than any of the others, and *P. alba* gave scarcely any distinct poplar odor, but rather the usual smell of the leaves of most plants kept under such conditions.   If the volatile substances which produce the odors were the stimulating factors, one might suspect that *P. deltoides* would develop intumescences on its leaves, which it never does; although, of course, it is not known that the same substances are involved in producing these different odors.

While there is a possibility that these volatile substances may in some way initiate the formation of the abnormal growths, it does not seem probable that this is the case.   Positive evidence that they are not the only stimulus was gained from the experiments in which a second set of leaves was introduced into a chamber in which one set had already been kept for some days.   These chambers always manifested a strong odor, but the second set of leaves did not develop intumescences, though the odor in the chambers was intensified by their presence.

The odors were equally noticeable in chambers from which the air had been exhausted by an aspirator and in those in which the oxygen tension was lowered by pyrogallol, and under these conditions intumescences did not appear.   It is possible that these volatile substances may be combined with a lowered oxygen tension in stimulating the leaves to abnormal growth, but it seems more likely that they are noticeable only because they are confined in the stagnant atmosphere and that they are only a part of an unusual complex of environmental conditions, but like carbon dioxid ineffective as a stimulating factor.

From the foregoing results it appears that oxygen is needed for the growth of intumescences, and that if too little of it is present an internal decline of the leaves results and no growth is possible.   On the other hand, the oxygen content of the air around the leaves must be lowered before the stimulus, whatever it may be, which initiates the intumescences can be developed.   The hypothesis which appeals to the writer as the most tenable of those which he has considered in connection with the data yielded by his experiments is that some product, or combination of products, of anaerobic respiration provides the required stimulus.

DISCUSSION

There is no doubt that intumescences and similar abnormal growths may be initiated by more than one type of stimulus. Haberlandt, Smith, Harvey and Rose, Doubt, Wallace, Von Schrenk, Douglas, and others have shown that chemical stimulation may give rise to such abnormalities, and the number of different chemicals used by these investigators is great enough to suggest that many others might produce similar effects.

Contrasted with the effect of chemicals studied by the above workers one finds a number of investigators who have believed that intumescences were initiated by physical factors. That direct injuries could cause intumescences on cabbage leaves has been shown by R. B. Harvey and Wolf, who studied frost injury and injury by wind-blown sand, respectively. The writer (13) found that punctures induced intumescences in leaves of *Hieracium venosum*.

Atkinson, Douglas, Dale, Viala and Pacottet, and Sorauer all have emphasized an excessive water supply and low transpiration as agents in the process. One must admit the possibility that these two components of the immediate environment of the plants were sufficient to cause these abnormalities in certain plants. In the present paper the writer has shown that neither excessive water supply nor reduced transpiration would cause intumescences in poplar leaves, nor would the two of them in combination effect this result. One is led to suspect that this may be true of some other plants in which the appearance of intumescences has been attributed mainly to excess of water. Steiner, for example, kept the leaves on which intumescences appeared in a damp atmosphere. It may be that this meant also that the air was confined around them. Douglas enclosed her experimental plants in bell jars and considered that the intumescences which appeared were due to excessive moisture. She made no mention of any provision for ventilation of the bell jars, and unless they were ventilated, the idea must be entertained that changes in carbon dioxid and in oxygen content may have had an important effect.

Küster found that when leaves of *Populus tremula* were floated on water the development of intumescences was greatest on the side of the leaf next the water. On the same side of the leaves the oxygen supply obviously would be most limited. Some, though not all, of Dale's experiments were made under conditions which would have allowed only a limited oxygen supply to the leaves. Likewise the water around the roots on which Hahn, Hartley, and Rhoads found lenticular outgrowths may have cut off the oxygen from the roots. The paper by Viala and Pacottet is too brief to give full details, but in it they state that intumescences on grape leaves were found immediately under the glass of greenhouses. The writer found in the course of the studies described above that even in ventilated chambers poplar leaves which were pressed closely together or against the walls of the chamber developed intumescences, while all the others remained

normal.   Perhaps the grape leaves which bore the abnormalities studied by Viala and Pacottet were not only close to the glass, but actually pressed against it in such a way as to form closed chambers.

Wisniewski and Schilling produced outgrowths on twigs by coating them with paraffin and vaseline.   These authors were not included among those who used chemical means of stimulation in producing abnormalities because it seems to the writer that these stimuli were physical rather than chemical.   In fact, Smith insists that all the chemicals by which he caused abnormal growths were physical rather than chemical in their effect. Schilling believed that lowered transpiration was the main cause of the response in the twigs which he studied, but he suggested that lowered oxygen tension also might have been concerned.   No other investigator, so far as the writer can determine from the literature on intumescences, has even referred to the possibility that deficiency of oxygen might serve as a stimulus to renewed growth in plant organs.   It is not unreasonable to assume that in some of the instances cited above, where the possibility of an oxygen deficiency was not eliminated, this factor may have been more important than the one most emphasized.

It is possible that the apparently diverse stimuli which result in intumescences are not really different in their final effect on the cells. Little is yet known as to the changes which are induced in the cells before they begin to increase in size, or to divide.   R. B. Harvey suggested that the growth stimulus in frozen cells of cabbage leaves was due to a partial precipitation of the proteins with a consequent increase in the permeability of the cells to water, and in the ability of the cells to hold sugars.   This hypothesis seems to agree well with the development of intumescences in poplar leaves in which the cells increase in volume but do not divide.   The precipitation of the proteins Harvey believes to be due to an increase in hydrogen-ion concentration in the affected cells.   He found such a change in pH in frozen cabbage leaves, although later (10) he discovered that the actual tumors on castor bean, beet, and *Bryophyllum* were less acid than the surrounding tissues.   Incomplete studies on the pH of poplar leaves kept in closed chambers indicate that the pH is lowered by this treatment.

The cause of the changes in the pH of poplar leaves may possibly be found in the products of the anaerobic respiration necessitated by the low oxygen tension in closed chambers.   Thomas (21) found that when the oxygen concentration of storage chambers falls below 10 percent the apples in these chambers begin anaerobic respiration and produce alcohol and acetaldehyde.   In apples the accumulation of acetaldehyde causes storage scald.   These, or other products of anaerobic respiration, such as oxalic acid or other organic acids, may account for a change in hydrogen-ion concentration in the cells, and ultimately for the initiation of abnormal growths.

At present no explanation can be given of the fact that a great many

species of plants do not produce intumescences when placed in closed chambers were deficiency of oxygen must soon check aërobic respiration (12). It is even more difficult to understand why only three species of poplars produce these growths, and all other species of the genus tested thus far fail to do so. A more intimate knowledge of the respiration of these plants in atmospheres deficient in oxygen may explain these differences in the behavior of plants so closely related.

SUMMARY

1. Leaves of *Populus grandidentata* and *P. tremuloides* produce intumescences under any condition which causes them to be surrounded by stagnant, moist air.

2. Submerged leaves do not produce outgrowths, apparently because of lack of oxygen.

3. Leaves in the open air remain normal even though constantly sprayed with water for a period of several days' duration.

4. Leaves injected with water under pressure do not develop intumescences in the open air, nor in dry air in closed chambers.

5. A moist atmosphere and an adequate supply of internal water are required for the production of intumescences. Injection of water under pressure does not increase the growth, or the number of intumescences on leaves surrounded by moist air.

6. Leaves kept in ventilated moist chambers do not produce intumescences.

7. Intumescences are not produced in closed chambers in which the air is kept dry.

8. An excessive water supply, internal or external, or both internal and external, does not initiate intumescences in poplar leaves.

9. An atmosphere free of oxygen, or very low in oxygen content, does not allow intumescences to develop.

10. The oxygen content of the air around the leaves must be reduced below that of normal air before intumescences are initiated. The extent of the reduction required has not been determined.

11. An accumulation of carbon dioxid in the chambers which contain the leaves is not required for the production of intumescences. Removal of all carbon dioxid from the chambers does not prevent the formation of these outgrowths.

12. It is suggested that some product, or combination of products, of incomplete oxidation, due to lowered oxygen tension, stimulates the leaf cells to renewed growth which results in the production of intumescences.

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17

THE TRANSITION REGION IN THE SEEDLING OF *RICINUS COMMUNIS:* A PHYSIOLOGICAL INTERPRETATION

F. MURRAY SCOTT AND HELEN M. SHARSMITH

INTRODUCTION

The orientation of the xylem and phloem conducting strands differs in the root and stem regions of the axis of vascular plants. The vascular continuity of the entire axis is maintained, however, by an area between root and stem, where rearrangement of the bundle elements occurs. This so-called " transition " from root to stem structure has been a subject of interest to the plant anatomist since the days when Gérard, and later van Tieghem, followed in detail the changing paths of xylem and phloem throughout this particular zone. The work of van Tieghem and the later morphologists is summarized by Compton (8). Three types of transition are described by van Tieghem, and a fourth is added by Sargant (17).

Some of the more recent investigators retain the descriptive terms of van Tieghem, and talk of the " splitting," " rotation," and " fusion " of the vascular bundles. Kean (15) describes the seedling anatomy of *Mesembryanthemum* in this manner. Eames and MacDaniels (11) summarize the four main methods of division, rotation, and reunion of the conducting strands, and refer to a " transition region where root and stem meet, and where the various parts of each organ merge into the other." Avery (1) identifies the seedlings of maize, oats, and wheat as representative of van Tieghem's three types of transition, but he considers these three to be probable variations of one main morphological type.

A somewhat deeper interpretation of the root-stem transition phenomena is introduced when the subject is approached from the standpoint of phylogeny. If the law that " ontogeny recapitulates phylogeny " is accepted, the first trace of vascular system to appear in the developing seedling is to be regarded as the prototype of the conducting system of vascular plants in general. The seedling has been followed through consecutive stages of growth by a number of different workers, and we find that the results of these investigations have been variously interpreted. The development of the vascular system is fully described by Chauveaud (6) and summarized in the " law of basifugal acceleration." In the root there occurs a primitive type of vascular pattern, which is gradually suppressed during the growth of the stem and replaced by a distinctly different arrangement. That is to say, the centrifugal structure of the stem, which results in the suppression or elimination of the original centripetal condition, is secondary and superposed

The position of the transition region in the seedling is then determined by the rate—i.e., degree of acceleration—at which the primitive centripetal protoxylem is replaced by the superposed metaxylem elements.   In the plant axis it is therefore found that root structure may be maintained upward throughout the hypocotyl, and even into the cotyledons; or the primary protoxylem may be replaced by centrifugal elements at the base of the hypocotyl.   Root and stem are thus regarded as fundamentally continuous, and differ only in that they represent different phases of vascular evolution.

The work of Chauveaud is corroborated and further extended by Dauphiné (9, 10), Bouvrain (2), and Tronchet (19).   During the progress of basifugal acceleration, Tronchet recognizes three stages of development, which he describes as the alternate, the intermediate, and the superposed phases.   The first, the alternate and primitive phase, always occurs in the roots of seedlings, and is characterized by the presence of centripetal xylem. The intermediate phase is usually attained in the region of the " collet," and here the vascular elements differentiate in a tangential direction, while the superposed phase is associated with stem structure, and is determined by its secondary centrifugal elements.

Chauveaud's theory of basifugal acceleration is disputed by Bugnon (3, 4, 5).   The latter attempts to show that the gradual destruction of the centripetal protoxylem in the hypocotyl, which to Chauveaud exemplifies slow basifugal acceleration, is merely the result of intercalary growth.   When it so happens that intercalary growth in the hypocotyl is negligible, then the transition from root to stem will take place rapidly, and this Chauveaud would interpret as a case of rapid basifugal acceleration.   Bugnon, however, apparently considers these structures in relation to present-day plants only and does not enter into a discussion of the phylogenetic significance of the existing vascular arrangements.

Another phylogenetic interpretation of seedling development essentially different from that of Chauveaud is given by Gravis (12).   Gravis emphasizes the fact that root and stem are distinct morphological entities, which are linked together in vascular connection in the transition region by means of certain distinct units of vascular structure, the " triads."   A triad consists of a small group of centripetal protoxylem cells lying between the two halves of a typical collateral bundle, the latter being derived from the cotyledon and the developing plumule.   The vascular pattern produced by the triads resembles in its essential features the plan of structure met with in certain fossil axes or rhizocauls.

Similar to the triads of Gravis are the " double bundles " of Thomas (18). The " double bundle " unit of structure, consisting of two separate collateral strands of phloem and xylem, between which lies a single group of centripetal protoxylem cells, is characteristic of the hypocotyl.   The seedling anatomy of about 150 species of dicotyledons is described by Thomas in terms of diarchy and tetrarchy, and the relation of the vascular strands to the planes of the

cotyledons is emphasized.   The relative primitiveness of these common di-
cotyledonous types is fully discussed, and it is suggested that a careful inter-
pretation of the phylogenetic relationships, indicated in the vascular connec-
tions of the transition regions, may be of taxonomic significance.

In the present paper, an attempt is made to regard the transition region
from a physiological rather than from a purely anatomical and phylogenetic
viewpoint.   The differentiation of tissues is considered in reference to the
supply of food materials and of water available for growth during the early
stages of germination.   To begin with, the digested food from the endosperm
is conveyed to the growing root tip through unspecialized meristematic tissue,
and thereafter, slightly later, along the developing procambial strands.   The
progressive growth of the radicle down into the soil is accompanied by ab-
sorption of water and salts, and the resultant upward current of water soon
becomes of definite physiological importance.   It will be seen that in the
seedlings of *Ricinus communis* the opposing streams of food and water con-
duction determine the paths of xylem and phloem differentiation, and may
therefore be considered as the principal factors in the causal anatomy of the
transition zone.

METHOD

About forty series of hand and microtome sections from seedlings of
various ages of *Ricinus communis* were made.   In each series sections were
cut from the base of the radicle to the top of the hypocotyl.   The hand sec-
tions of fresh material were stained with phloroglucin and hydrochloric acid
for easy identification of, the lignified vessels, and the microtome slides of
embedded seedlings were stained in safranin and light green.   The distribu-
tion of the vascular elements in relation to the other tissues was followed from
the root upward to the stem, and the changes in the region of " collet " were
especially noted.   Detailed descriptions will be given of seedlings in three
stages of development: first, a very young seedling, seven millimeters in
length, in which lignification of xylem elements is just beginning; second, a
slightly older seedling, ten millimeters long, in which all the essential changes
of transition can be followed; third, a thirty-millimeter seedling, in which
the metaxylem is well developed and the radicle shows characteristic root
structure for the greater part of its length.

OBSERVATIONS ON THE SEEDLING OF RICINUS COMMUNIS

In all the fully differentiated seedlings examined, the arrangement of the
vascular tissues at the base of the cotyledons is the same.   Eight collateral
vascular bundles, with centrifugal xylem and centripetal phloem, occur here
in a typical stem arrangement, no variation of the octarch condition being
found.   When completely developed the young root or radicle is consistently
exarch and possesses alternate centripetal xylem and phloem strands, with
tetrarch symmetry in the majority of cases.   (In a few seedlings pentarchy

was noted, but this somewhat unusual condition will not be discussed at this time.)

In following out the gradual development of this adult condition through consecutive stages of growth, we see, first of all, that at the beginning of germination the young radicle and hypocotyl emerge as a minute cone of meristematic cells. At this early stage, therefore, food is conveyed through undifferentiated tissue to the developing root apex. As growth proceeds, the vacuolation and expansion of the differentiating pith and cortical cells demarcate the remaining meristematic tissue. The latter persists as a cylinder leading from the base of the cotyledons to the root tip, supplying both the root apex and the intervening tissues with the necessary food materials. Continuation of meristematic activity, accompanied by cell expansion and elongation in all tissues, leads to the development of procambial strands within the meristem cylinder, and the passage of food is now concentrated mainly in these four channels. At this stage of development, absorption, and presumably thereafter upward conduction of water, begin to assume their rôle in the economy of the seedling. Vascular differentiation, as it occurs in the seven-millimeter seedling, now commences. In a seedling of this length, the anatomical description of which follows, the phases of development already outlined may be clearly traced.

Seven-millimeter seedling

Cross-sections of the radicle tip of this seedling show, as in all roots, a terminal meristem of small, undifferentiated, actively dividing cells. Passing upward from the root apex, differentiation of pith and cortex and the consequent demarcation of the meristem cylinder are next apparent (text fig. 1). This is immediately followed by the development, within the cylinder, of four well-marked procambial strands. It is at this level, about three millimeters above the root tip, that the first protoxylem elements are laid down (text fig. 2). They occur in four groups, situated in tetrarch symmetry on the inner periphery of the meristematic cylinder, adjacent to the procambial strands. Each xylem group contains but two or three annular tracheal elements, which are differentiated in a centripetal direction toward the center of the axis as in a typical older, tetrarch root.

Above this initial protoxylem, the identity of the procambial strands is less evident, for cell division suddenly accelerates between them, with the result that four new interradial meristematic strands are now apparent. Such increase in meristematic activity, right and left of the original procambial strands and protoxylem groups, implies a diversion of food materials toward these areas and gives to the pith a sinuous outline (text fig. 3). Since this diversion occurs immediately above the protoxylem, it is occasioned, in all probability, by the increasing water supply which follows vascular differentiation. Here, where the upward water current induces a change in the path of the downward food stream, lies the future position of the transition region.

As we trace the development of the axis to a still higher level, we see that the four new interradial strands become increasingly prominent in the meristem cylinder. Thereafter they begin to split lengthwise (text fig. 4); but, even in the upper region of the hypocotyl at this stage, the separation is still incomplete. The eight contiguous wedges thus formed indicate the position of the collateral bundles that will be differentiated at this level in an older seedling (text fig. 5).
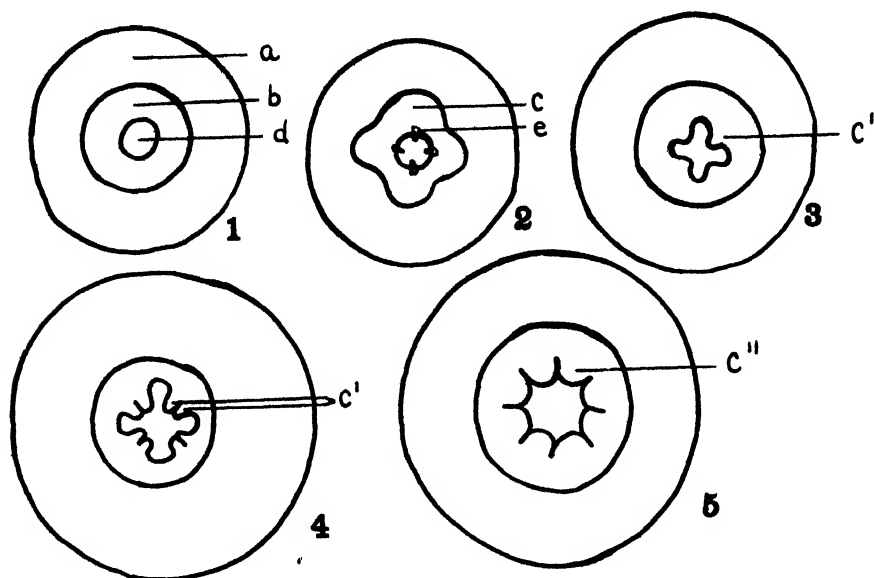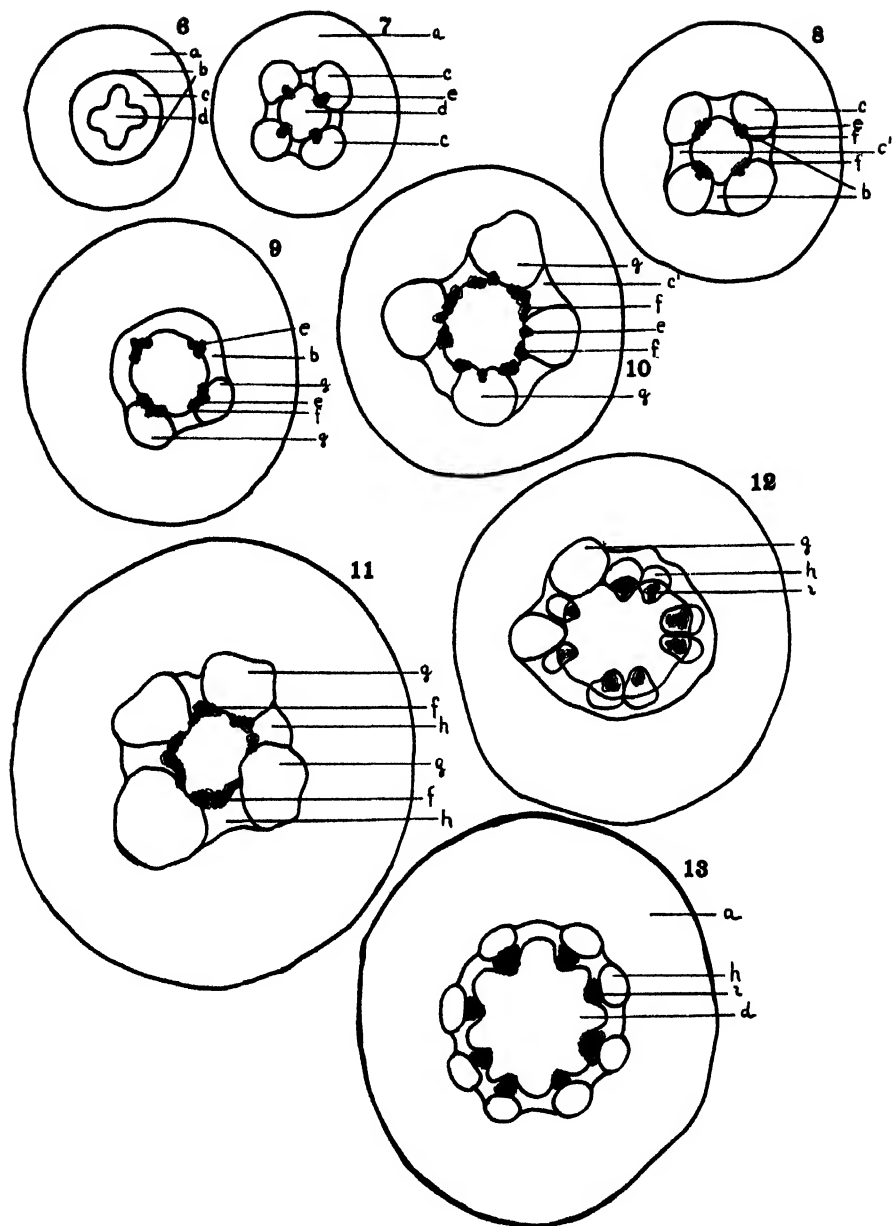

TEXT FIGS. 1-5. The relation of protoxylem elements to procambial in the seven-millimeter seedling. For explanation, see text. *a*, cortex; *b*, procambial cylinder; *c*, procambial strand; *e*, protoxylem; *c″*, interradial protoxylem strands.

Ten-millimeter seedling

In the ten-millimeter seedling, lignification has proceeded to a greater extent, and the root-stem transition is now more easily followed. Lateral roots are just beginning to develop, but so far they have not emerged on the surface of the root. Just as in the younger seedling, a meristem cylinder is found above the root tip, and four prominent procambial strands of actively dividing cells are again seen (text fig. 6). About three millimeters back of the root tip, cross-sections show four groups of centripetal protoxylem, similar to the tetrarch arrangement described in the younger seedling. Each of these groups of protoxylem contains but three to six lignified elements (text fig. 7), adjacent, as described in the previous case, to the procambial paths of food conduction (text fig. 14).

A cross-section just below the level of the "collet" (where root and hypocotyl merge) shows the beginning of metaxylem differentiation. The metaxylem cells are laid down internally to the protoxylem, with the result

Tеxt Figs. 6–13. This series of diagrams was drawn to the same scale (camera lucida, × 40) and illustrates the anatomy of the ten-millimeter seedling as seen in transverse section at various levels. For full explanation, see text. *a*, cortex; *b*, procambial cylinder; *c*, procambial strand; *c'*, interradial procambial strand; *d*, pith; *e*, protoxylem; *f*, tangential metaxylem; *g*, lateral root; *h*, phloem; *i*, centrifugal metaxylem; *k*, crushed xylem elements.

that each of the four groups of centripetal xylem is now composed of seven to twelve lignified elements (text fig. 8). With this further lignification, the upward current of water continues to assume greater significance in the growth of the seedling, and, as in the younger stage, diverts, to a certain extent, the streams of food material to channels intermediate with the procambial strands. This is indicated by an increase in meristematic activity in these intervening areas, with a consequent increase in diameter of the entire meristem cylinder. These intervening areas mark the lines of future phloem differentiation at a higher level.

Concurrent with the gradual alteration in position of the main paths of food conduction, the focus of lignification changes, and metaxylem is now formed on either side of the original protoxylem. This is clearly seen in the next sections, where the oldest (smallest) and most external protoxylem cells of each centripetal xylem group form a distinct and isolated patch. Right and left of the latter, and separated therefrom by a few undifferentiated cells, two strands of metaxylem have developed in a more or less tangential direction, the oldest and smallest elements being nearest the isolated protoxylem. This tangential tendency is more evident at a slightly higher level, and the lateral metaxylem strands now form an interrupted ring of lignified cells adjacent to the internal margin of the meristem cylinder (text figs. 9, 10, and 15).

Branch roots may begin their development at this level, arising from the meristem cylinder on the same radii as the four original procambial strands and the protoxylem of the lower radicle. Development of lateral roots entails accumulation of food material and consequent meristem activity. At this stage of growth, also, cell expansion is general in cortex, pith, and meristematic tissues, and results in a crushing of the first-formed protoxylem cells (text figs. 10 and 16). In the development of the lateral roots, the downward passage of food is blocked at certain points in the meristem cylinder, and now the main paths of food transfer to the root tip become definitely located to right and left of the emerging secondary roots. In these four areas, between the four original procambial strands, the first phloem differentiation becomes evident, and sieve tubes and companion cells can be discerned. Xylem formation now takes place in a less tangential and more centrifugal direction, radially toward this external differentiating phloem, and thus each lateral xylem strand appears arc-shaped in transverse section (text fig. 11). As growth and development continue, the original proximity of these cells to the protoxylem is lost, for the lateral xylem strands now begin to show continuity along radii between the original protoxylems, finally pairing into four new groups. Further development shows completion of this tendency; the original protoxylem cells disappear and four collateral bundles are now present, each consisting of centrifugal xylem and centripetal phloem separated by a narrow tangential area of meristematic tissue (cambium). At this level secondary roots cease to be formed between the vascular bundles,
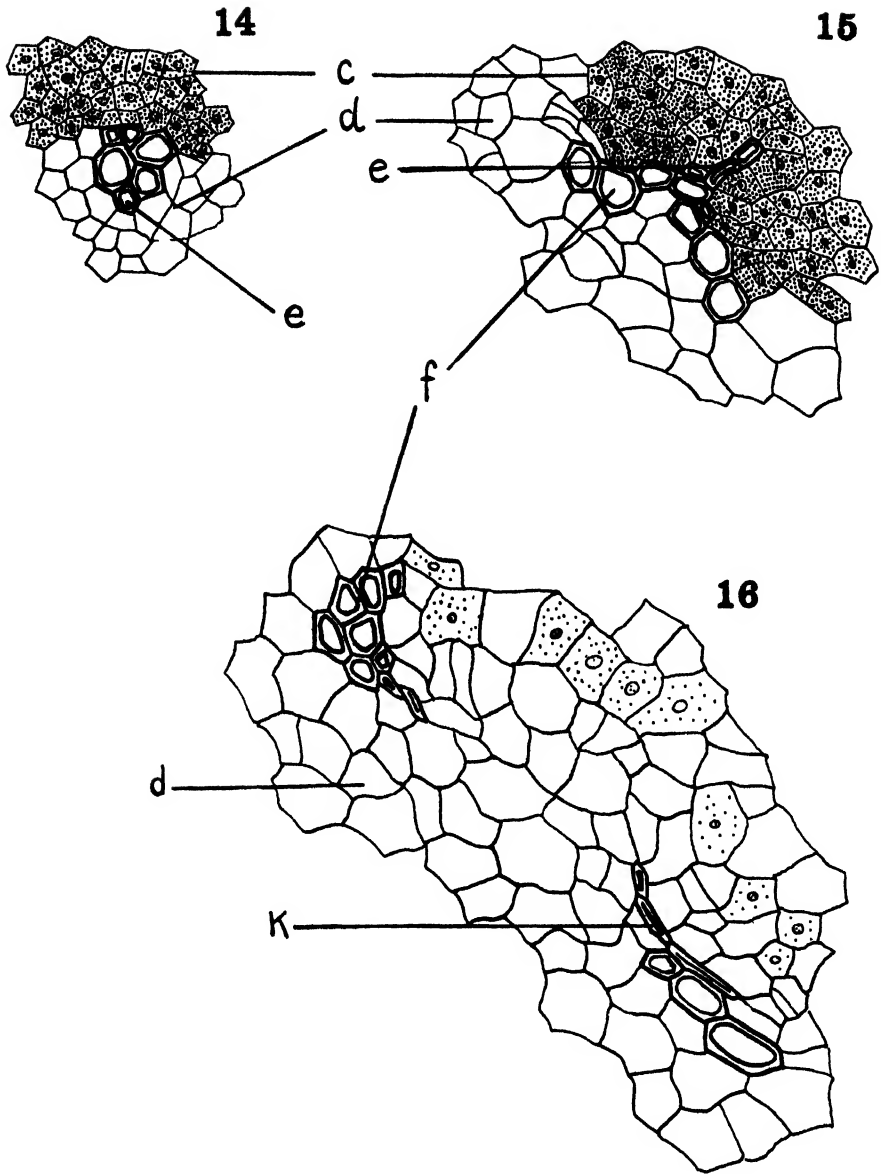
TEXT FIGS. 14-16. Details of previous figures. ×440. FIG. 14, cf. figures 2 and 7. FIG. 15, cf. figures 8 and 9. FIG. 16, cf. figures 10 and 11.

and in their place is seen the beginning of the development of medullary rays and interfascicular cambium. In seedlings of this age, therefore, typical stem structure is demonstrable in the upper half of the hypocotyl. Further concentration of the upward water current is now established, and the downward passage of food is definitely localized in the specialized phloem tissue

of each vascular bundle. Above this, still additional change occurs in the distribution of the vascular tissues. In the next sections, each of the four collateral bundles just described shows the beginning of a central line of lengthwise radial splitting (text fig. 12), comparable, perhaps, to the diversion of the xylem strands already described in the transition region. A short distance upward, the longitudinal split in the bundles results in their complete separation. Thus in the upper extremity of the hypocotyl the characteristic stem arrangement of eight symmetrically placed collateral bundles is found (text fig. 13).

Thirty-millimeter seedling

In the older seedling of *Ricinus* the hypocotyl has elongated, and below the ground a very marked development of secondary roots has taken place. The radicle, as before, is clearly differentiated into the usual zones: meristematic root tip, the region of elongation (root-hair zone), the region of differentiation, and the mature zone.

There is no marked difference between younger and older root in the tip, in the region of elongation, or in the region of differentiation. In the mature zone, however, the primary, centripetal xylem and phloem elements are fully formed at the expense of the original cylinder of meristem, and as a consequence the tetrarch and alternate arrangement in this region of the radicle is much more pronounced. With the increase in length and the development of the radicle to give this typical and " normal " distribution of the root tissues, the relative volume of the food-conducting strands is greatly decreased in comparison with that of the younger seedling, where, as we have seen, the four prominent procambial strands of the meristem cylinder occupy a relatively large area in the radicle.

At the upper end of the radicle, in the lower region of the hypocotyl, the secondary roots arise in acropetal succession. The beginning of this lateral root zone approximately coincides with the level of the transition region. The tissue strains, which are here involved in meristematic division and intercalary growth, partially crush the original protoxylem, and profuse development of metaxylem tends to complicate further the former simple transition outline. It is still possible, however, to trace the fundamental change from centripetal to centrifugal xylem, just as it is found in the younger seedling. The temporary persistence of the protoxylem poles, coupled with the increased number of xylem elements in these larger seedlings, is responsible for the definition, at this level, of transition structures comparable to the " triads " of Gravis and the " double bundles " of Thomas. The distribution of the vascular tissues in the upper part of the hypocotyl, above the transition area, is essentially the same in the younger seedlings, and the vascular strands differ only in the relative increase of the number of elements.

<center>DISCUSSION</center>

The growth of the root-stem transition region in the young seedling is of course a continuous process. In it, however, there may be recognized certain well-marked and distinct phases of development, described as the alternate, the intermediate, and the superposed phases. These terms refer to the relative orientation of the xylem and phloem, and are more or less self-explanatory. As the previous descriptions show, all three phases of growth are met with in the early stages of germination of the castor-bean seedling. Moreover, they appear in succession—that is to say, in ontogeny the alternate phase marks the first beginnings of vascular differentiation, while later development results in the formation of the intermediate and superposed phases.

If we regard the seedling from the physiological rather than from the anatomical and phylogenetic standpoint, then we must attempt to trace the origination of xylem and phloem strands in relation to food conduction and water absorption, the main factors concerned in the growth of the plant. Already in the ungerminated seed, traces of future venation are outlined in the delicate tissues of the cotyledon. When digestion and food conduction begin, we can therefore readily understand that the incipient current of food materials from the endosperm reserve will tend to be concentrated along certain more or less well-defined paths, predetermined during the formation of the seed. These paths appear to be the developing procambial strands of the young radicle.

In a meristematic tissue, the actual increase in protoplasm involved in the multiplication of cells points to protein metabolism as the dominant physiological function of that region (16). Differentiation, with the vacuolation and expansion of cell contents, and the accompanying increase in cell wall surface, implies a slowing down of protein synthesis, and the gradual onset of carbohydrate metabolism. Localized excess of carbohydrate metabolism results in the thickening of the cell wall, which marks the initial stage of lignification.

In the castor bean, therefore, we may suppose that, at a certain stage of growth, conditions arise in the developing axis, such that carbohydrate metabolism is activated on the inner margin of the procambial food-conducting strands, at four distinct foci—viz., the foci of lignification. Meantime the expansion of the cells prevalent in all tissues denotes an increase in absorption, which in turn entails an upward current of water. This current of water flows more freely along the differentiating xylem elements. The result is that the downward stream of food is diluted and at the same time diverted from its main concentration in the procambial strands to the interradii of the meristem cylinder, where later the phloem elements are developed.

That the foci of lignification originate in a definite spatial relation to the food-conducting strands, as is indicated by their first appearance, seems to receive additional confirmation here; for the process of lignification is like-

wise diverted tangentially along the interradii—that is to say, in the direction of the diverging stream. As the interradial food conduction becomes relatively stabilized, so also does the process of lignification, and the metaxylem lignification foci remain stationary along the interradial arcs. The direction of development ceases to be tangential and becomes definitely centrifugal, and the youngest xylem elements are as heretofore in proximity to the food stream.

At about the same time, also, the differentiation of the phloem elements external to and on the same radius as the metaxylem is gradually completed. The conduction of food materials becomes mainly concentrated in the differentiated phloem; while of the original meristematic cylinder there eventually remains but a single layer of meristematic cells, the actively dividing cambium cylinder.

SUMMARY

1. A brief survey of previous work on the anatomy of the transition region is given.

2. An attempt is made in the present paper to interpret the development of the transition region from a physiological standpoint. The differentiation of the xylem and phloem strands is therefore considered in relation to the paths of food and water conduction in the young seedling.

3. On the inner margin of the procambial (food-conducting) strands, four centers of lignification are determined, in which originate the tetrarch and centripetal protoxylem strands. Increasing water absorption and the consequent upward conduction along this protoxylem serve to dilute and to divert right and left the downward stream of food materials. The foci of lignification are likewise diverted tangentially right and left of the protoxylem.

4. As the food stream becomes relatively stabilized in its new interradial position, the foci of lignification follow suit, and the centrifugal metaxylem is now laid down.

5. No explanation for the initial tetrarch arrangement of the foci of lignification is offered, other than that the physiological conditions at these four points are influenced by the direction of the incipient food streams, the path of which is, in its turn, predetermined during the formation of the seed.

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THE RELATION OF THE SATELLITES TO THE NUCLEOLUS IN *GALTONIA CANDICANS*

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INTRODUCTION

Since S. Nawashin reported the presence of " Trabants " or satellites borne by one pair of the chromosomes of *Galtonia candicans* Des. in 1912 (10), a mass of literature has appeared which describes these structures in many species of plants. In most cases a single pair of chromosomes bears satellites, but in some species two or more pairs are involved. The satellites in *Galtonia* were considered by Nawashin (10, 11) as structural units of the chromosomes concerned, perhaps as their terminal chromomeres. He distinguished between the " Leitkörperchen," the small knob found on each chromosome beyond the subterminal spindle-fiber constriction, and the " Trabant " or satellite. The satellite is a small chromatic mass attached by a long, fine thread to the proximal segment or " Leitkörperchen " of each chromosome of one pair. This thread is, therefore, distinct from the subterminal spindle-fiber constriction. Newton (12), however, concluded that the satellites of *Galtonia* differ from the small proximal segments beyond the spindle-fiber constrictions in other chromosomes only in that the satellites are more widely separated from the chromosomes. Thus the fiber bearing each satellite is the result of an attenuation of the chromosome at the region of spindle-fiber attachment.

Nawashin (11) reported that during the prophases of somatic divisions the satellite chromosomes of *Galtonia* are in contact with the nucleolus by their satellites. Newton (12) did not describe this relation. Sorokine (15) reported a pair of chromosomes with satellites in *Ranunculus acris*. She concluded that the satellites are present in the resting nucleus, each attached to the nucleolus by a fine thread. As the cell prepares for division, the nucleolus divides and each new nucleolus retains a satellite. In the late prophases both nucleoli become satellite chromosomes. Senjaninova (14), studying the same species, found that the satellites are attached to the nucleolus in the resting nucleus but are " picked up " by a pair of chromosomes in the prophases of the somatic divisions and at diakinesis in the prophases of the heterotypic division. She found also that the satellites disappear at the metaphases of the heterotypic division but are present during the anaphases and telophases. In a later paper Sorokine (16) figured the satellite chromosomes of *R. Chius* attached to the nucleolus at diakinesis.

McClintock (6) first reported the presence of a pair of satellite chromosomes in *Zea Mays*. The fiber bearing each satellite is distinct from the spindle-fiber constriction. Later (7) she described the pair of satellite chromosomes attached to the nucleolus during the open spireme stages of the heterotypic division. Sometimes the place of contact with the nucleolus is at a short distance from the satellites, the chromosomes then being enlarged at the points of contact. In other cases a bud is constricted from the nucleolus, and the fibers bearing the satellites surround the base of the bud. Cooper and Brink (1) have shown that the pair of satellite chromosomes is involved in a ring of four chromosomes in the semisterile-5 race of corn. The chromosomes of this ring form a cross in the open spireme stage, one arm of the cross being attached to the nucleolus by the satellites.

Sax (13) described the attachment of one pair of chromosomes of *Callisia* to the nucleolus from the open spireme stage to late diakinesis. He did not describe them as bearing satellites, but concluded that at all stages it is the same chromosome pair that is thus located.

S. Nawashin (10, 11) also described distinct races of *Galtonia* differing in the size of the satellites. If the pair of satellite chromosomes has satellites of like size, the plant belongs to a " symmetrical race." The satellites of a race of this type may be either large or small. If one chromosome of the pair has a large satellite and the other a small one, the plant represents an " asymmetrical race." Thus the species is described as " dimorphic " or " polymorphic " with regard to the satellites. Nawashin found that the asymmetrical race occurs more frequently and assumed that the symmetrical races are less viable.

Species of *Muscaria* (11), *Crepis* (8, 9), *Vicia, Leontodon,* and *Thalictrum* (5), *Ranunculus* (14, 15), *Hordeum* (3), and *Rumex* (4) have also been described as polymorphic with reference to satellites borne on one pair of chromosomes. Although the terms applied to the various races by these authors differ in some instances from those given by Nawashin, the basis on which the races are differentiated is essentially the same. Most of the descriptions of such races have been based upon studies of the chromosomes in somatic tissues. Emme (3), studying thirty-eight varieties of *Hordeum,* found a number of these varieties to be polymorphic with regard to the presence or absence of one or both satellites of a particular pair of chromosomes. Most varieties show no satellites, a few show one, and still fewer show both satellites present. He suggested that there may be a factor borne on the satellite that is lethal in the homozygous condition. Medwedewa (8) made genetical studies of *Crepis dioscoridis* to determine the behavior of the satellites. The three races of this species described by M. Nawashin (9) were tested by Medwedewa for their constancy. One race is homozygous for large satellites, one homozygous for small satellites, and one heterozygous. The homozygous races breed true, while the heterozygous race yields the three types in a 1 : 2 : 1 ratio. The race with large satellites is much more vigorous

than the one with small satellites, while the heterozygous race may be intermediate or may vary toward either extreme. Medwedewa correlated the vigor of the race homozygous for large satellites with the greater chromatin content of the nuclei.

MATERIAL AND METHODS

Bulbs of *Galtonia candicans* were obtained from Theodore Payne Company, Los Angeles, and from Peter Henderson and Company, New York. There is no apparent difference as to the chromosome structure and behavior as between the plants grown from these two lots. The meristematic regions of root tips sprouted in water were used for the study of the somatic divisions and the anthers of flowers produced by bulbs grown in soil for the meiotic divisions. Nawashin's fixative gave the best results for root tips. The anthers were treated for about forty-five seconds with Carnoy's solution (60 cc. of absolute alcohol, 10 cc. of glacial acetic acid, and 30 cc. of chloroform) and then with Flemming's medium solution for twenty-four hours. Sections were cut 5 to 14 microns in thickness and stained with Heidenhain's iron-alum haematoxylin.

OBSERVATIONS

The somatic divisions

The diploid chromosome complement of *Galtonia candicans* consists, as described by S. Nawashan (11) and Newton (12), of four pairs of long chromosomes, two pairs about half as long, and two pairs of short chromosomes. The satellites are borne on one of the pairs of medium length, referred to by Nawashin as the "X-chromosomes." Newton studied the points of spindle-fiber attachment for the different chromosomes. My observations agree with Newton's with respect to all the chromosomes except the pair bearing satellites. All chromosomes, including this pair, exhibit a subterminal constriction. The degree of separation of the small proximal segment from the chromosome varies considerably. In some cases, especially in one of the pairs of long chromosomes, this separation is sufficiently conspicuous to suggest the presence of satellites. The distinctness of the proximal segment is more obvious during the early prophases and late anaphases. During the metaphases the spindle-fiber constriction is usually not conspicuous. Each of the pair of satellite chromosomes shows a subterminal spindle-fiber constriction in addition to the fiber bearing the satellite (pl. 5, figs. 1, 4, 5). Thus the satellite-bearing fiber does not mark the point of spindle-fiber attachment as Newton (12) believed; my observations on this point are in harmony with those of S. Nawashin (10, 11).

One or two nucleoli are present in the resting nucleus. In the early prophases the satellite chromosomes are not distinguishable. As the chromosomes become denser and more chromatic, the satellites can be recognized. Thus it is not until the middle prophases that it is possible to distinguish between the pair of satellite chromosomes and the other pair of medium length.

When the satellite chromosomes first become recognizable, a satellite is definitely attached to each chromosome of the pair (fig. 1). The relation of the satellites to the nucleolus at this time varies in different nuclei. One of the satellites is in contact with the nucleolus and the other free (fig. 1), or both satellites are free from the nucleolus (fig. 2). No nuclei were observed at this stage in which both satellites were in contact with the nucleolus. One of the satellite chromosomes is always found near, but not necessarily in contact with the nucleolus.

In some instances each satellite divides in the prophases just before or at the time that the double nature of the attached chromosome becomes noticeable (figs. 2, 3). During the late prophases and the equatorial-plate stage, the spindle-fiber constrictions are not so evident as in the early prophases. As the daughter chromosomes separate, however, these constrictions become evident (fig. 4). The daughter satellites apparently remain close together or in contact until after the separation at the region of spindle-fiber attachment. In the anaphases the satellite is usually directed toward the equatorial plate, but in some cases it is directed toward the pole (fig. 5). A like behavior was observed in *Scilla nutans* by Darlington (2) and interpreted as evidence that the movement of chromosomes to the poles is governed by some attraction operating over the entire chromosome and not localized at the point of spindle-fiber attachment. In *Galtonia* the point of spindle-fiber attachment appears as a narrow, lightly staining region in some anaphase chromosomes. In others, however, the region of spindle-fiber attachment stains darkly, and a definite constriction is present (fig. 5).

It is not possible to follow the satellites through the telophases. The relation of the internal structure of the chromosomes to the satellites was also not determined. The chromonemata do not appear in preparations stained darkly enough to show the satellites. This is also generally true of the chromosomes in the meiotic divisions.

The meiotic divisions

Only one nucleolus is present in each microspore mother cell after the last pre-meiotic division. The satellite chromosomes first become recognizable in the prophases of the heterotypic division when the leptotene strands begin to pair side by side at the start of synizetic contraction. The satellites are found paired and both in contact with the nucleolus at this time (fig. 6).

During synizesis the chromatic strands usually conceal the nucleolus, but occasionally they contract to one side of it. In the latter case the strands of the pair bearing the satellites extend from the synizetic knot to the nucleolus (fig. 7). Thus the contact between the satellites and the nucleolus is maintained during synizesis. As the strands first become loosened from the contracted condition, the two satellites of the pair are somewhat apart from each other but both in contact with the surface of the nucleolus (fig. 8).

In some cases the nucleolus has a bud similar to that described in the pollen

18

mother cells of corn by McClintock (7). The relation of the satellites to the nucleolar bud is variable. Sometimes the satellites and their fibers completely girdle the bud at the constriction between the bud and the nucleolus (fig. 9a). In other instances the satellites and their fibers only partially encircle the base of the bud (figs. 9c, 9d), while in still others the two satellites lie in the constriction at one side of the bud (fig. 9b). Not all the nucleoli show buds; some retain a smooth outline throughout the open spireme stages. The satellites in such nuclei are either in contact with or somewhat apart from each other, but both are in contact with the surface of the nucleolus.

The subterminal spindle-fiber constrictions become visible during early diakinesis. The constrictions are at approximately the same distance from the proximal end of each chromosome as they are in somatic nuclei. When the nucleolus lacks a bud, the satellites, lying on the surface of the nucleolus, are either in contact with each other or somewhat separated (figs. 10, 11a). Buds which are smaller than many of those observed during the open spireme stages appear on some of the nucleoli (fig. 11b). It is assumed that these buds are formed at this time on nucleoli which did not develop a bud during the earlier stages.

During diakinesis the fibers connecting the satellites with the corresponding chromosomes gradually contract so that sometimes a satellite is not distinguishable on one or both of the homologues (fig. 12). For the most part, however, the satellites are visible until the nucleolus disappears. Great variation is found in the relation of the satellites to the nucleolus during diakinesis The satellites may be separate and in contact with the surface of a smooth nucleolus; or they may be separate, each satellite lying at the base of a bud from the nucleolus; or the satellites may be in contact with each other, partially encircling a bud from the nucleolus (fig. 13).

The satellite chromosomes shown in figure 14 have separated at their proximal ends, and one chromosome has moved away from the nucleolus. Attached to the other chromosome is a heavy girdle surrounding a small nucleolar bud. Since no satellite is visible on the chromosome seen in end view, and the girdle seems too large to represent a single satellite, it may be that the satellites have failed to disjoin. Such an occurrence might lead to a condition in which the two satellites are borne on one chromosome, such as was found by Taylor (17) in the root tips of one bulb of *Allium cepa.* It is also conceivable that a satellite might become lost or so intimately associated with its mate that only one chromosome of the pair would show a satellite. The result would be a race of *Galtonia* asymmetrical with respect to the presence or absence of the satellites. Such a race might show the breeding behavior described for a heterozygous race of *Crepis dioscoridis* by Medwedewa (8).

In many pollen mother cells the nucleolus and its bud become completely separated in late diakinesis. In most such cases the pair of satellite chromosomes remains attached to the bud (or new nucleolus; fig. 15). The satellites may then be widely separate or may lie close together in contact with the

former bud (fig. 16). In a few instances one chromosome is found in contact with a relatively large nucleolus, while the other chromosome of the pair is in contact with a smaller nucleolus (fig. 17). It is possible that the two nucleoli in such a case have resulted from the division of a single nucleolus; possibly the smaller nucleolus arose as a bud from the larger one during the open spireme stages. If so, the two satellites were probably separate on the surface of the nucleolus as in the case shown in figure 13.

The satellites cease to be recognizable in late diakinesis at about the time of disappearance of the nucleoli. The satellites apparently become fused with the corresponding chromosomes in consequence of the shortening of the attaching fibers. The satellites remain unrecognizable throughout the heterotypic anaphases and telophases.

The appearance of the satellites in the homoeotypic division is, except for two points, essentially similar to that in the somatic divisions. Since the halves of each chromosome remain separate from the anaphases of the heterotypic division, their attached satellites are separate when they are first distinguishable in the homoeotypic prophases (fig. 18). The nucleolus also apparently disappears rather early in the prophases of the homoeotypic division. No definite relation could be determined between the satellites and the nucleolus before the disappearance of the latter. During the anaphases both the satellite fibers and the spindle-fiber constrictions are evident. In one case an anaphase chromosome apparently had two satellites borne on a single fiber (fig. 19). This condition may have resulted from the division of the satellite during the anaphases. Usually no evidence of such division is found at this time.

All the evidence accumulated in this study is decidedly against the occurrence of recognizably " symmetrical " and " asymmetrical " races in the material studied. Since the apparent size of an object so small as a satellite of *Galtonia* is determined in part by the depth of stain, comparisons between the satellites appearing in different preparations would obviously be misleading. Even in a single preparation it is not possible to eliminate completely the factor of unequal staining. In any one preparation many variations are to be found in the sizes of the satellites. Many of the illustrations presented here have been chosen to show this variation. Figures 1 and 2, showing somatic chromosomes, were made from the same preparation. In figure 1 the satellites are very different in size; in figure 2 they are approximately equal. The variation is even greater in the heterotypic prophases. Figures 9c and 9d, for example, were drawn from pollen mother cells of the same anther sac. Figures 11a and 11b were taken from a single preparation. The chromosomes shown in figure 16, from another plant, were likewise found in one preparation. Figures 12, 13, and 17 are also from one preparation. With the amount of variation shown here, it can hardly be assumed that races constant with respect to the size of the satellites, whether " symmetrical " or " asymmetrical," occur in this material of *Galtonia candicans*.

SUMMARY

1. Satellites are borne by the chromosomes of one pair of medium length in *Galtonia candicans*.

2. Each satellite is carried on a fiber that is distinct from the constriction at the point of spindle-fiber attachment.

3. During the somatic prophases at least one of the pair of satellite chromosomes is found near the nucleolus; in many cases the satellite is in contact with the nucleolus.

4. During the heterotypic prophases the satellites are regularly in contact with the nucleolus from the leptotene stage until the time of disappearance of the nucleolus.

5. One or two buds appear on the nucleolus at different stages of the heterotypic division. If one is present, the satellites appear in the constriction at the base of the bud, either encircling or lying at one side of the bud. If two are present, a satellite is found in the constriction at the base of each bud.

6. The fibers bearing the satellites gradually shorten during diakinesis, and by the time the nucleolus has disappeared the satellites are apparently fused with the chromosomes which carry them.

7. The nucleolus disappears rather early in the homoeotypic prophases. No relation could be determined between the satellites and the nucleolus during these stages.

8. The material studied did not show distinct races or forms with satellites of the same or different sizes.

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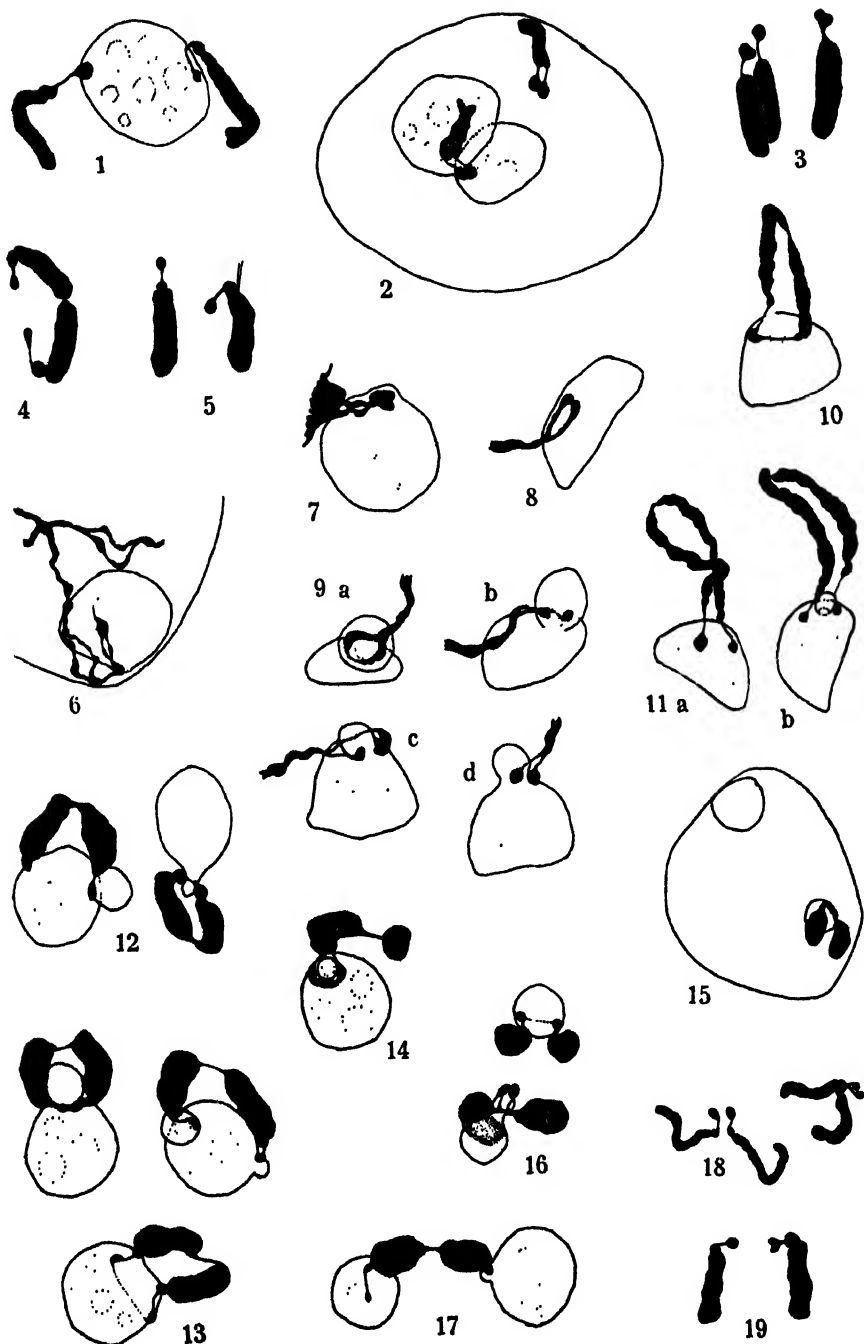
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EXPLANATION OF PLATE 5

All drawings were made with an Abbé camera lucida at table level. For all except figure 15 a Zeiss 2 mm. apochromatic objective, N.A. 1.3, and a Zeiss 18 × compensation ocular were used, giving a magnification of about 3900 diameters. For figure 15 a Zeiss 12 × compensation ocular was used, giving a magnification of about 2400 diameters.

SOMATIC DIVISIONS

FIG. 1. Middle prophases. The smaller satellite is in contact with the nucleolus, while the larger one is at some distance above.

FIG. 2. Late prophases. The shaded chromosome is below both nucleoli and the satellites of this chromosome are close to but not in contact with the lower nucleolus.

FIG. 3. A pair of satellite chromosomes just before they move on to the equatorial plate. The two halves of one chromosome are clearly recognizable.

FIGS. 4, 5. Satellite chromosomes during the anaphases.

MEIOTIC DIVISIONS

FIG. 6. At the beginning of the synizetic contraction.

FIG. 7. During synizesis.

FIGS. 8, 9. During the open spireme stages.

FIGS. 10, 11. The pair of satellite chromosomes at early diakinesis.

FIGS. 12–14. At late diakinesis.

FIG. 15. At late diakinesis. The bud to which the satellite chromosomes are attached has become separate from the old nucleolus.

FIG. 16. Separated nucleolar buds with satellite chromosomes in contact.

FIG. 17. Two nucleoli, possibly the result of the separation of a large bud; one member of the pair of satellite chromosomes attached to each nucleolus.

FIG. 18. Early and median prophases of the homoeotypic division.

FIG. 19. Homoeotypic anaphases. One satellite has apparently divided.

THE DECOMPOSITION OF CERTAIN TYPES OF FOREST LITTER UNDER FIELD CONDITIONS

JOSEPH G. FALCONER, J. W. WRIGHT, AND H. W. BEALL

INTRODUCTION

Observations in the northern forested regions of America and Europe and at higher elevations in the Southern Appalachians and the Central Alps show that a thick mat of slowly decomposing forest litter is commonly found in closed coniferous stands on the forest floor. It was not until the last few years of the nineteenth century that litter decomposition was given serious study, and even now our knowledge of this problem is in many respects imperfect. More recent results from investigations in microbiological laboratories justify the statement that litter accumulation tends to occur wherever temperature and moisture conditions are such as to retard the activity of microörganisms.

The relation of temperature and of moisture as measured by rainfall to the quantitative decomposition of forest litter in pine stands of east-central Ontario, Canada, was studied in 1929 and 1930. The results of this investigation are given in this paper.

The economic value of information relating to the decomposition of litter is apparent when we consider the problem of forest establishment through natural regeneration. Barr (1929) recognized the necessity for some form of litter treatment prior to seeding, if naturally regenerated spruce seedlings in British Columbia were to survive the first season. Hesselman (1926) made an intensive investigation of litter accumulations in the forests of Sweden. He found that nitrification processes in the soil were very essential for the proper establishment of Scots pine and that certain types of forest litter retarded the activity of nitrifying organisms.

Literature dealing with the influence of temperature on microbial processes has been discussed by Waksman (1931, p. 741–752). It appears that the temperature for optimum development differs for the different organisms. From the point of view of moisture the problem is more complex. But as a broad generalization, excess moisture favors anaerobic activity, whereas the optimum moisture content for aerobic activity is to a large degree dependent upon the coarseness or fineness of the soil medium (Waksman 1931, p. 544, 548).

Experimental evidence showing the relative rates of litter decomposition for different coniferous species under natural forest conditions is very inade-

quate. What information is available appears to indicate that the lignin complex is very resistant to decomposition (Waksman, 1931, p. 400, 598; Falck, 1926, 1927).

Watson (1930) investigated the decomposition of needles collected from white (*Pinus Strobus* L. and *Pinus resinosa* Ait.) and red pine in central New England. According to his analysis, 24.8 per cent of the freshly gathered red pine needles and 31.8 per cent of the white pine needles constitute a lignin complex which is very resistant to decomposition. At the end of the period a second analysis was made. The figures show that materials other than the lignin complexes had been reduced. The red pine needles lost 20 per cent and the white pine 16.4 per cent of the original oven-dry weight over the two-months' period. Considered from a relative point of view, it appears that a lignin complex makes up the greater percentage of the accumulation products resulting from the decomposition of red and white pine needles under laboratory conditions.

Melin (1930) conducted work of a similar nature to that of Watson (1930). In so far as his investigation paralleled Watson's work, the results are similar. He found that 21.89 per cent of the original oven-dry weight of white pine needles was composed of a lignin complex.

FIELD EXPERIMENTS

Description of the areas studied

The field experiments were carried out in naturally regenerated stands of *Pinus Strobus* L., *Pinus resinosa* Ait., and *Pinus Banksiana* Lamb., at the Petawawa Forest Experiment Station. This station is located on the Petawawa River, a tributary of the Ottawa in eastern Canada.

The climate of the region is favorable to forest growth. The growing season is relatively long and the annual mean temperature is high in comparison to most northern forested regions. The climatic data given in tables 1 and 2 were collected on the forest where the experiment was carried on. The following data—collected at Pembroke, Ontario, a station about 25 miles from the forest, by the Dominion of Canada Meteorological Department throughout the period 1866 to 1927—indicate the general climatic conditions of the region:

Mean annual precipitation .... 34.7 inches
Mean annual temperature .... 40.9° F.
Mean length of growing season .... 127 days
Mean temperature of growing season .... 62° F.

The region is an upland of moderate relief. The slightly undulating skyline, the rounded physiographic features, the striated boulders and rock faces, and the terminal moraines furnish abundant evidence that the area was at one time covered with ice.

Stratified sands and gravel, glacial drift, and rock material weathered in

19

situ form the parent soil. Under the combined influence of the various factors present in the region the soil profile development is tending toward the " Sandy Podzol," which has been described by Glinka (1927). A brief description of the soil profile of the white pine site is tabulated below.

Soil sampled on the white pine site

Hori-zon	pH [1]	Weight [2]	Thickness in inches	Moisture equivalent [3]	Ignition loss [4]
F	4.95	5376	1.3	—	—
H	4.85	28761	0.8	—	—
A₂	4.95	—	2.0	4.56	1.73
B	5.00	—	13.0	4.31	3.16
C	5.42	—	24.0+	2.65	—

[1] The pH was measured by means of a potentiometer and quinhydrone electrode.
[2] Air-dried weight in kilograms per acre.
[3] Moisture equivalent calculated in per cent of oven-dry weight at 105° C.
[4] Ignition loss calculated in per cent of oven-dry weight.

Forest conditions appear to vary according to the nature of the parent soil material and the local climatic influences. The hardwoods are more common on the sites where the parent material was weathered in situ, whereas *Pinus strobus* L., *Pinus resinosa* Ait., and *Pinus Banksiana* Lamb. occupy soils derived from glaciated material, especially the sands and gravels of glacial origin.

The most abundant and frequent species among the lesser vegetation in the Jack pine stands are *Vaccinium canadense* Kalm., *V. pennsylvanicum* Lam., *Epigaea repens* L., *Gaultheria procumbens* L., *Myrica asplenifolia* L., *Coptis trifolia* (L.) Salisb., and *Dicranum* (sp.?) ; in the red and white pine stands, *Cornus canadensis* L., *Aralia nudicaulis* L., *Maianthemum canadense* Desf., and *Oryzopsis asperifolia* Michx.

The present investigation

From June to September, 1929 and 1930, litter decomposition was studied in situ, under even-aged pure stands in the 40- to 60-year age class. During the field season of 1929 the study was confined to white pine and bracken (*Pteris aquilina* L.), the latter being used because of its apparent resistance to decomposition. In 1930 the study was extended to include the decomposition of litter in red and Jack pine forests. Climatic data were collected over the period of the investigation.

Experimental technique. Rectangular-shaped galvanized iron wire baskets of about 5 mesh to the inch were used as containers. The bottom of each basket was covered with cheesecloth. A portion of the litter the same size and shape as the basket was cut around, removed, and placed within. This permitted close contact of the litter with the mineral soil, yet retained the material within the basket. The oven-dry weight [5] of the litter was calculated by sampling.

[5] Oven-dry weight taken at 105° C.

Twigs of the pines were collected from the ground where they had fallen. These were placed in baskets similar to those described above. After oven-drying, the baskets and their contents were set on the surface of the litter. A similar treatment was ascribed to the stems of bracken.

The oven-dry weight was determined at the end of the season for all samples. The loss in weight over this period was attributed to decomposition processes.

RESULTS

The effect of rainfall and temperature upon the decomposition of forest litter. The rainfall data are given in table 1.

TABLE 1. *Rainfall throughout period of experiment*

Month	Year	
	1929	1930
	Inches	Inches
June......	1.31	4.78
July....	1.89	3.71
August......	1.24	2.65
September......	1.31	3.68
Total.......	*5.75*	*14.82*

The rainfall for the four months in 1930, as given in table 1, is nearly three times the rainfall of 1929. This increase in rainfall was accompanied by an increase in temperature. The average mean temperature· for 1930 is 63.65 as compared to 59.85 degrees for 1929. The mean monthly temperatures throughout the period the experiment was conducted are given in table 2.

TABLE 2. *Temperature throughout period of experiment* [6]

Year	Month	Mean maximum	Mean minimum	Mean
1929	June......	70.3	48.8	59.55
	July......	76.8	50.6	63.70
	August......	71.0	49.2	60.10
	September......	68.2	43.9	56.05
	Mean for period......	*71.6*	*48.1*	*59.85*
1930	June......	76.0	55.9	65.96
	July......	75.5	54.1	64.78
	August......	74.1	55.3	64.71
	September......	66.1	48.1	67.11
	Mean for period......	*73.5*	*53.8*	*63.65*

[6] Temperature in degrees Fahrenheit.

·The difference in rainfall and temperature as shown by tables 1 and 2 should affect the activity of the microörganisms—that is, if field conditions

are in any way analogous to laboratory conditions. The following table (table 3) brings out this relationship.

TABLE 3. *Forest litter decomposition* [7]

Description of material	Weight in grams [8] at:		Loss	
	Beginning of season	End of season	In grams	In per cent
1929				
White pine				
F and H layers [9]	240	223	17	7.1
Twigs < ¼″ in diameter	537	511	26	4.8
Bracken (Pteris aquilina L..)				
Stems	200	189	11	5.5
1930				
White pine				
F and H layers	570	493	77	13.5
Twigs < ¼″ in diameter	407	376	31	7.6
Bracken				
Stems	143	130	13	9.1
Red pine				
F and H layers	331	292	39	11.8
F and H layers	327	278	49	15.0
F layer	129	124	5	3.9
Twigs < ¼″ diameter	384	372	12	3.1
Jack pine				
F and H layers	231	216	15	6.5
Twigs < ¼″ diameter	498	489	9	1.8

[7] The period included is from June to the latter part of September.

[8] Data are based on equilibrium at 105° C.

[9] The terms F and H are adopted from Hesselman (1926). They have been further explained by Morgan (1931). The letter F is used to designate the fermentation layer, that portion of the organic accumulation in the process of decomposition still exhibiting the characteristics and structure of the original material from which it is derived. The letter H is used to designate the humification layer, that portion of the organic accumulation which lost the characteristics and structure of the original material from which it was derived. These two layers constitute the "soil cover" of Glinka (1927), and Shaw (1927) describes the material of these layers as "accumulated organic debris found upon the soil surface."

From table 3 it is obvious that the quantitative decomposition of samples of similar material was greater in 1930 than in 1929. In the case of the combined F and H layers, the loss of the former year exceeded that of the latter by 6.4 per cent. A similar relation existed with reference to the twigs and the bracken. The loss in 1930 exceeded that of 1929 for the twigs by 2.8 per cent, and the bracken lost nearly twice as much weight in 1930 as in 1929. This increase in decomposition may be attributed to the increase in rainfall, the rise in temperature, or to both. It is worthy of note at this time that factors which influence decomposition processes in the laboratory also appear to influence decomposition in the field. If the lignin content of the different materials be considered, there is a further significance attached to the data given in table 3.

The effect of the lignin complex upon the decomposition of bracken and white pine. The principal source of the materials from which the F and the H layers in the white pine stands are derived may be traced to the pine needles. It is obvious from table 3 that in both years, 1929 and 1930, the bracken lost less weight than the combined F and H layers of the white pine. This is attributed to the higher lignin content of the former as compared to the latter. This difference has been shown by Melin (1930). The justification for this deduction is further substantiated when the chemical constituents of white and Jack pine are compared.

The effect of chemical constituents on the decomposition of Jack pine and white pine wood. The Forest Products Laboratory of the United States Forest Service, Madison, Wisconsin, has determined the chemical constituents of white and Jack pine wood. These analyses are shown in table 4.

TABLE 4. *Chemical analysis of Jack and white pine wood*

Materials recovered and reagents used	Per cent		Excess in favor of white pine
	Jack pine	White pine	
Lignin	29.9	27.5	−2.4
Total cellulose	58.3	60.0	+1.7
Alpha cellulose	42.8	44.0	+1.2
Total pentosans	14.0	10.6	−3.4
1 per cent NaOH solubility	13.9	16.2	+2.3
Alcohol benzol solubility	4.2	6.9	+2.7
Hot-water solubility	3.1	4.6	+1.5
Ether solubility	2.35	2.9	+0.55
Hydrolysis number	20.3	5.6	—

It is obvious from an inspection of the data in table 3 that the wood, in the form of twigs less than one-half inch in diameter, of the three different pines decomposes at different rates under like conditions. Jack pine twigs lost 1.8 per cent, red pine 3.1 per cent, and white pine 7.6 per cent over the period studied. Unquestionably, the difference in the rate of decomposition of the wood from these pines is to be ascribed to their difference in chemical composition as revealed by table 4.

From the data given in table 3 one would infer that because Jack pine is more resistant to decomposition than the wood of white pine: (1) The hot-water-soluble products of white pine would exceed those of Jack pine. (2) The lignin content of white pine would be less than that of Jack pine. To a large degree table 4 confirms these statements. White pine contains 4.6 per cent of hot-water-soluble products as compared with 3.1 per cent in Jack pine. The lignin content of Jack pine exceeds that of white pine by 2.4 per cent.

Thus, from the point of view of chemical composition, it is clear why Jack pine is more resistant to decomposition than white pine. The wood of the former pine contains, as shown by chemical analyses given in table 4, a

smaller percentage of readily soluble materials (hot-water-soluble products) and a larger percentage of material soluble with difficulty, such as the lignin complexes, than the wood of the latter.

SUMMARY

This investigation was a study of the loss by weight of stems of bracken and the litter on the forest floor in a white pine stand during the field season of 1929. The study was continued throughout the field season of 1930 and was extended to include forest litter in stands of red and Jack pine. Brief descriptions of the soil and forest conditions have been given. The weather conditions over the two field seasons varied widely. During 1930 both the temperature and the precipitation were higher than in 1929. The following tentative conclusions are presented:

1. Under like conditions of temperature and precipitation: (a) The litter in a white pine forest was found to decompose more rapidly than the litter composed of the stems of the common bracken. (b) The litter of both red and white pine was found to decompose more rapidly than the litter of Jack pine. (c) The twigs of white pine were found to decompose more than twice as rapidly as those of red pine and over four times as rapidly as those of Jack pine. (d) The twigs of red pine were found to decompose more than 1.7 times as rapidly as those of Jack pine.

2. The loss in percentage by weight for all materials studied was found to increase with an increase in temperature and precipitation. Thus, a mean temperature of 63.65° F., when accompanied by a total precipitation of 14.82 inches, was found to be more favorable to the decomposition of the combined F and H layers in a white pine forest and the stems of the common bracken than a mean temperature of 59.85° F., when accompanied by a precipitation of only 5.72 inches.

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THE MORPHOLOGY OF THE DEVELOPING FRUITING BODY OF *LYCOPERDON GEMMATUM*

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Since no work has been done on the morphology and the development of the fruiting body of *Lycoperdon gemmatum* with the exception of that of Rehsteiner (1892), who studied the gross morphology and chiefly the older stages, it seemed advisable to investigate in detail the successive stages of the development of young basidiocarps from the minute primordia to forms with basidia. Interesting problems arose such as the origin of the basidiocarp primordia, the differentiation of the hyphae, the origin and differentiation of the glebal cavities, the binucleate condition, and the presence of hemispherical pads. This led to an investigation of the rhizomorph in connection with the formation of the fruiting body primordia.

HISTORICAL

The rhizomorphs of the Hymenomycetes and the Gasteromycetes have been found to be highly differentiated and specific for each species (de Bary, 1884; Bambeke, 1901; Hein, 1930). In 1884 de Bary summarized and compared the structure of the rhizomorph in a number of forms, including the Lycoperdaceae. The rhizomorph is composed of a central larger portion, the medullary zone, which is made up of a felted mass of tough hyphae running longitudinally and of unequal thickness, and a cortex of loosely interwoven hyphae. The medullary zone of the rhizomorph of *Lycoperdon gemmatum*, according to Rehsteiner (1892), contains two types of cells: the wide-lumened cells (10–25 μ in diameter), and the small, thin cells (2–3 μ in diameter); while the cortex consists of thin hyphae (2 μ in diameter) with very thick walls and narrow lumina which have adhering to them mycelial outgrowths of thin-walled, richly septate, often anastomosing hyphae.

As early as 1842 Tulasne compared and contrasted *Lycoperdon, Bovista*, and *Scleroderma*. The various layers of the peridium were noted and a superficial survey was made of the gleba in the older stages. This was followed by the work of Bonorden (1857), who contrasted *Lycoperdon* and *Bovista* on the basis of the morphological characters.

The most complete investigation of the morphology of *Lycoperdon laxum, L. gemmatum*, and several other Gasteromycetes was made by Rehsteiner in 1892. He followed the developmental gross morphology from the young stages through the mature stage but presents only one figure. The fruiting body primordia were first found to be noticeable as clavate-shaped swellings.

either terminal or lateral, on the thin rhizomorph. The youngest studied (.5 mm. long and .7 mm. broad) already showed differentiation of the radial hyphae at the periphery, but the rest of the fruiting body was a homogeneous structure of thin-walled hyphae. The peridium differentiates first and comes to maturity very early in comparison with the glebal cavities. Though the peridium early is composed of a definite region of radially directed hyphae, it is later differentiated into two regions: the outer pseudoparenchymatous layer of enlarged cells and the inner compact zone of radially arranged hyphae. Warts or markings are formed by the splitting and tearing apart of the cells of the outer region. The inner peridium is formed after the glebal differentiation has been completed. According to Rehsteiner the origin of the gleba is marked by the appearance of isodiametric spaces surrounded by the palisade-like compact hyphae with swollen ends. The origin and development of the cavities is varied and irregular. Continued differentiation of the cavities and the formation of new fertile ones take place at the top and sides of the glebal region and of the sterile cavities at the base. The labyrinth-shaped fertile cavities surrounded by tramal plates with basidia lining the openings become distinguished from the regularly shaped sterile cavities bounded by the palisade tissue. Further development involves the growth of the elements already present, followed by the decomposition and degeneration of all the glebal parts until finally only the capillitium and spores remain. The development of the opening for spore dispersal is described.

From a study based on a series of twenty stages, from 2 mm. in diameter to maturity, Cunningham (1927) described the general development of the fruiting body of *Lycoperdon depressum* and found it similar to that described for *Lycoperdon gemmatum*, with the exception of the formation of the diaphragm.

Hemispherical pads have been observed commonly on the cross walls of many of the Ascomycetes and Basidiomycetes (Strasburger, 1884; Harper, 1902; Levine, 1913; Hein, 1930).

Levine (1913) summarized the observations concerning the binucleate condition among the Basidiomycetes. Maire (1900) reported binucleate condition in the subhymenium and young basidium and spores of several species of *Lycoperdon*, including *L. gemmatum*. Nichols (1905) noted the binucleate cells in the rhizomorph of *L. pyriform*, while Cunningham (1927) described binucleate basidia in *L. depressum* but was unable to make out clearly the condition of the cells in the fruiting body.

<center>MATERIAL AND METHODS</center>

The fruiting bodies of *Lycoperdon gemmatum* used in this study were found in close clumps on a log. Pieces of the wood bearing the basidiocarps were cut out so as to disturb the material as little as possible. Flemming's medium was found to be a very satisfactory fixative. While the basidiocarps were in seventy per cent alcohol, they were teased apart and adhering particles

20

of the dirt and wood were removed with a camel's hair brush. In many cases pieces of the rhizomorph were left attached to the fruiting bodies and also tiny basidiocarps in contact with the older ones. Longitudinal and cross sections were cut at 7, 5, and 3 μ. Heidenhain's iron-alum haematoxylin with cotton-blue or erythrosin as a counter-stain gave good results for the nuclear condition and general structure of the fruiting body, while safranin and light green were especially good for the hemispherical pads and the structure of the rhizomorph.

<center>OBSERVATIONS</center>

The young rhizomorph is composed of three regions: the large compact central core, a narrow dark-staining fibrous zone surrounding the first, and an outermost loose cortex.

The central region in the young rhizomorph is made up of actively growing hyphae, termed "building" hyphae (fig. 1, pl. 6), which have thin walls, moderately dense protoplasm, binucleate cells, and which range from 2.5–4 μ in diameter. Cross walls with hemispherical pads are abundant, and the individual cells vary from 20–40 μ in length. The hyphae branch sparingly and are arranged parallel to each other and to the longitudinal axis of the rhizomorph in a compact crowded manner, though there is a slight amount of intertwining of the larger groups of the hyphae over and under each other.

The thick-walled, curved, twisted, irregular-shaped, unbranched "fiber" hyphae (fig. 2, pl. 6) are wrapped round the central zone in an intertwined loose arrangement. The lumen of each cell is narrow and in many instances contains rows and small groups of dark-staining granules, while the nuclei are not visible.

The hyphae of the cortical region resemble the "building" hyphae, except for the fact that they are smaller, more delicate, contain less dense protoplasm, and branch more frequently. The active binucleate cells have abundant cross walls, hemispherical pads, thin walls, and average in diameter 2–2.5 μ. Frequent cases of lateral anastomosing were found (fig. 16, pl. 7). The hyphae grow in all directions, intertwine individually with each other, and are very loosely arranged.

The growth of the rhizomorph is centrifugal, the new "building" hyphae being added to the outside of the central region inside the fiber region. By differentiation of the "building" hyphae new "fiber" hyphae arise, and in the cortex there is a general pushing outward and division of the cells. Very early in the development of the rhizomorph differentiation of the "building" hyphae takes place in the center. The first sign of this differentiation is that of an increase in diameter of the cells followed by the appearance of small granules, a less dense protoplasm, and non-visibility of the nuclei (fig. 15, pl. 7). The differentiation is not uniform, occurring by the individual cells of the individual hyphae rather than by groups, and proceeds centrifugally.

The typical "conducting" hyphae are formed as a result of the progres-

sive swelling and enlarging of the individual cells, the crowding, pushing, and lateral anastomosing, and the dissolving and disintegration of the protoplasm and cross walls, with the accumulation of the crystalloids and granules. Differentiation continues to a high degree, and the types of cells and arrangement and structure of the rhizomorph are specific for the species. However, fruiting bodies are formed on the young rhizomorph before much differentiation has taken place. In fact, at the base of the fruiting body the central region continues to be composed of only the " building " hyphae.

The fruiting body primordia were first noticed as minute spherical bodies on the upper surface of the rhizomorphs. As the thin, greatly branched rhizomorph was dissected out of the earth or teased from near the surface of a log, these small knob-like swellings were found sparsely scattered along the rhizomorph, being soft, white, and in close proximity to the structure from which they have arisen. They more frequently arise from the rhizomorph at the base of an older basidiocarp and become pressed close to it. Many compact groups of immature basidiocarps of various ages were found, with the youngest in the middle, which have arisen on the rhizomorph in between the older fruiting bodies.

The primordia of the basidiocarps are either lateral in position on the rhizomorph or terminal on the small branches. Among the twenty very young stages studied twelve were lateral and four terminal in position and the other four were proliferated out of the older basidiocarps (fig. 20, pl. 8).

Several rhizomorph strands may play a part in the formation of one basidiocarp. Evidences of the result of such fusion were seen. One form, measuring 1.1 mm. by 2.5 mm., was directly connected with two strands of rhizomorphs, while another one, slightly older (1.3 mm. by 1.9 mm.), had three connections. It would seem as if the primordia, while in the homogeneous stage, or at least before differentiation had progressed very far, came in contact with each other. The " building " hyphae which make the fundamental tissue of the fruiting body were growing very rapidly and interlocked. As the two primordia expanded, the hyphae became interwoven and intertangled more intimately until there was a fusion into one fruiting body.

The youngest basidiocarp found measured .22 mm. by .37 mm. This had been torn from the rhizomorph, but another one .3 mm. by .5 mm. was found connected with the rhizomorph (fig. 18, pl. 8). The basidiocarp primordia are formed by the " building " hyphae of the central region of the rhizomorph. A group of such hyphae change from the longitudinal course and as a unit grow at a right angle to the other " building " hyphae of the rhizomorph. They break through the fiber and cortex regions and immediately begin more rapid growth and rearrangement. Branches become abundant, and these and the original hyphae together grow radially outward in all directions, soon interweave and intertangle very complexly, wind around, and form a spherical knot on the rhizomorph. The broken regions of the fiber and cortex may be seen forming a sort of ring at the base of the basidiocarp. On the periphery

of the knot at the side or top may be seen groups of the broken and dying cells, which are no doubt cells of the cortex broken off and carried along by the homogeneous mass.

Basidiocarps may proliferate out of others, usually older. These appear as small knot-like swellings on the side of the fruiting body. They may be near the base or in a median position. In longitudinal and cross sections the arising basidiocarps show a direct connection with the primordia of the inner peridium of the older fruiting body (fig. 20, pl. 8). This primordium of the inner peridium is an undifferentiated region made up of the " building " hyphae which have the same arrangement as the homogeneous basidiocarp primordia. This region is bounded on the outside by the exoperidial primordium of closely compacted cells, parallel to each other and extending radially outward, with swollen tips, while toward the inside of the homogeneous zone is the region of developing glebal cavities. A group of " building " hyphae become parallel to one another and grow outward as a unit more rapidly than the surrounding hyphae. These either break through the exoperidial region, or the exoperidium was never differentiated over this point. When the outside is reached, the " building " hyphae radiate outward in all directions, interweave, and intertangle to form the new basidiocarp in the same manner as the rhizomorph forms the fruiting bodies. The basidiocarps from which new ones arise were of different sizes and in different stages of development but always had the undifferentiated region. One of these (1.1 mm. by 1.5 mm.) contained very few cavity primordia, while in the other (1.5 mm. by 2.3 mm.) greater differentiation had taken place in the center.

The earliest primordia (fig. 18, pl. 8), as mentioned before, are perfectly homogeneous throughout, made up of hyphae of similar structures which are binucleate, have hemispherical pads, fairly dense protoplasm, thin walls, and are dividing very rapidly. Abundant cross walls are found, and the hyphae branch frequently. They grow irregularly radially outward from the lower center in all planes, intertangle, and interweave intricately. Even the tips of the hyphae at the edge turn back and grow so that the whole appears as a sphere or a ball with a fairly smooth edge. At first this is a rather tight, compact, rounded structure, but as growth continues it expands outward and becomes loose in arrangement. As development proceeds, the loosening continues and the peripheral development is very marked. Growth is general and uniform throughout the whole structure. Though the hyphae are still interwoven and intertangled with each other, they are not so intricately or compactly in contact as before. The cells of the hyphae average $3–4\,\mu$ in diameter and range in length from $20–40\,\mu$, tending to be longer in the central region of the body. As a proof of the rapid expansion of the hyphae, the protoplasm is less concentrated and stains lightly. The ends of the hyphae at the periphery of the sphere tend to become free and point outward (fig. 20, pl. 8). This starts first at the top of the ball and gradually progresses toward the base.

The very first differentiation is one of arrangement only and takes place at the periphery. Here the hyphae, all the ends now being free, assume a more radial direction and in a palisade formation. The cells divide more rapidly at the tip, resulting in short cells. Again such development originates at the top of the fruiting body primordium but rapidly takes place all around. The structure is still composed of one type of hyphae and in the center has the same arrangement as in earlier forms but slightly looser.

One basidiocarp of this age measured .4 mm. by .6 mm., while others studied were .6 mm. broad by from .6 mm. to .8 mm. long. There is a tendency for the increase to be slightly greater in length than in breadth.

The peripheral hyphae now become definitely radial, parallel, and in more intimate contact with each other. The septation has progressed so that each hypha now consists of a row of short cells. The outermost cells are slightly swollen, become clavate or tubular in shape, and show slightly different color reactions because of the lack of protoplasm. This region, the primordium of' the exoperidium, can easily be distinguished from the central region, extending for 60 μ in depth. A number of the fruiting bodies at this stage were studied and averaged in size from .75 mm. by .75 mm. to .9 mm. by .9 mm. Others were slightly longer, being .66 mm. by 1.5 mm.

Basidiocarps of the size and age first mentioned vary somewhat in their internal structure. The majority of them had the homogeneous structure so loosely arranged that the lighter portions were present, while a few had irregular dark portions (fig. 20, pl. 8). These as seen in cross sections were knots or intimate groups of more narrow hyphae with shorter cells and quite dense protoplasm. They were in intimate contact with each other, tangled, and twisted in groups, which were scattered in the central part of the developing fruiting body. This is the first sign of the differentiated hyphae that will later be described in connection with the formation of the palisade lining the glebal cavities. It seems that the development is not the same in all cases, and it may be that this variation is due to the influence of the environment and contact with other developing forms.

The present observations concerning the differentiation and development of the exoperidium agree with those described previously by Cunningham (1927) for *Lycoperdon depressum* and by Rehsteiner (1892) for *L. gemmatum*. The exoperidium has the two zones: the inner of the radial hyphae, parallel to each other, very compact with partial gelatinization of the walls, made up of short, binucleate cells with hemispherical pads on the walls; the outer of the inflated swollen tips becoming pseudoparenchymatous in nature, in some cases taking little or no stain because of the absence of the protoplasm and nuclei, while in other cases staining irregularly dark because of groups and masses of granules of disintegrating products of the protoplasm.

However, no correlation of the development of the exoperidium with the size of the fruiting body and the central differentiation was found in the literature. At the time when the peripheral hyphae are definitely swollen

and becoming pseudoparenchymatous, the basidiocarp is from .6 mm. to .9 mm. broad by 1 mm. to 1.5 mm. long. In the center the first few cavity primordia are present, as shown by figure 19, plate 8. As the exoperidium shows more definitely the two regions, pseudoparenchymatous and radial, the cavity primordia are greater in number but still irregular in shape (fig. 21, pl. 8). While the central part is filled fairly well with cavity primordia in forms 1 mm. to 1.5 mm. broad by 2 mm. long (figs. 22, 23, pl. 8), the splitting of the outer region of the exoperidium, with fraying and dying cells, is taking place. It will be noted that the basidiocarp primordia are spherical (figs. 18, 20, pl. 8), that early developing stages are nearly spherical (fig. 19, pl. 8), but that the older stages with the formation of the glebal cavities present are longer in proportion to their width (figs. 21, 22, 23, pl. 8). The sizes and shapes of the basidiocarps vary because of the environment and contacts.

The first glebal cavity primordia were noticed in forms measuring .6 mm. broad by 1 mm. long. The cavity primordia were very few in number and were seen only in a few median sections in the central part. However, in several younger stages averaging .9 mm. by .9 mm. looser areas were noticed. Detailed study with greater magnification revealed the developing cavities. Great variation was found among the early cavities, disclosing several types of origin.

In general, however, there first appear irregular lighter places in the undifferentiated tissue. These portions are made up of the same type of hyphae as the tissue surrounding, but less closely interwoven. The spaces are long and very irregular. They progress in every direction, a few being parallel to the longitudinal axis of the fruiting body, others parallel to the radial axis, but most of them directed from the inside toward the outside at various angles to the axes. There seems to be no definite arrangement with relation to one another except that they are fairly close together and limited to the central region. These less closely woven parts, no doubt, have arisen by an unequal growth and irregularity of cell division of the hyphae in the central part as the general outward growth and expansion toward the periphery are taking place.

Next a tearing apart of certain hyphae in these looser portions occurs (fig. 3, pl. 6), which results in cracks and fissures. In the openings, broken hyphae are found with frayed, hard, torn edges, while other hyphae which are narrower, with longer cells that are seemingly stretched, extend across the cavity (figs. 3, pl. 6; 13, pl. 7; 26, 27, pl. 8). The splits are peculiarly shaped. Very few are straight, but seem to be variously curved, rounded, and even angled. They vary greatly in size and extent. Some of the cracks involve the whole length of the looser area, while others show a progression starting with a small part and gradually involving the whole. On the other hand, two small openings may merge together, giving one long fissure. It is of peculiar interest that this cleavage or splitting is a continual progressive process depending on the force of the pulling of the hyphae in different parts resulting from the uneven growth.

A still greater pulling of the rapidly growing cells, combined with the tension of the less active groups of cells surrounding the cavity, causes the cavity primordia to widen. More torn and frayed hyphal ends appear and signs of the plasmolysis and degeneration of these exposed broken cells are seen (figs. 13, pl. 7; 27, pl. 8). Short sections of broken hyphae and small masses of degeneration products are free in the cavity. A number of the cells of the fundamental hyphae cross the opening (figs. 3, pl. 6; 27, pl. 8). Some of these are narrow, long-celled, and stretched, while others are swollen and irregular in outline. The walls are thinner and the protoplasm is very vacuolate. The tips of these enlarged cells are even more swollen and the walls are extremely thin.

As a result of the breaking, stretching, gelatinization, and disintegration of the hyphae, all signs of the fundamental hyphae in the cavity disappear (fig. 4, pl. 6). Combined with this is the elongation; and the cavity, opening with a smooth outline, is lined by very compact palisade. Some of the cavities, sterile, become round or oblong, while others, fertile, are irregularly curved, lobed, and elongated (figs. 22, 23, pl. 8). However, signs of the broken hyphae and a few hyphae of the undifferentiated structure crossing the cavity may persist for a long time. Cavity primordia completely lined with palisade may have a few of these strands crossing them. Again this process is progressive. Several small spaces may merge into one cavity or a cavity may broaden, followed by a cracking and tearing at one end which results in an extension of the cavity.

As the openings are developing, groups of differentiated hyphae arise. These develop irregularly through the undifferentiated tissue but in close proximity to the cavity primordia (fig. 3, pl. 6). The newly formed hyphae are first noticed in intimate groups of very closely intertangled hyphae that are turned in all directions. The hyphae themselves are narrower, of shorter cells, and filled with very dense protoplasm, so that they take a deeper stain. These hyphae later form the palisade lining the cavities. There seems to be no regularity in the origin of the groups, but there are scattered, intimately interwoven knots which are few at first but later increase in number and size.

From their tangled position the differentiated hyphae radiate toward the opening, the free ends extending into the cavity (fig. 13, pl. 7). Cell division is very rapid and a row of three or four very short cells 4–5 μ in length is cut off at the tip, with the two nuclei of each cell often arranged parallel to the cross wall close to each other (figs. 8, 11, 12, pl. 7). The hyphae become arranged parallel to form a pallisade. Others which are narrower push into this crowded region and become parallel to those already present (fig. 10, pl. 7). Longitudinal walls are flattened against each other and a compact tissue is formed (figs. 11, 12, pl. 7).

The ends are irregularly arranged at first, some farther into the cavity than others, probably because of unequal growth and different ages of the hyphae (figs. 5, pl. 6; 13, pl. 7). Subsequent growth causes the evening up of these ends until the contour of the cavity is regular.

The formation of the palisade is progressive, gradually increasing and following the breaking and splitting apart of the fundamental tissue. Many cavities are found with palisade at certain portions compactly arranged, but at other portions only lined with irregular broken ends and knots of palisade primordia (figs. 13, pl. 7; 27, pl. 8). As the palisade becomes continuous, the cavity becomes enlarged and on the inside has an even contour. There follow further growth of these palisade cells, the pushing in of others, a more crowded, compact arrangement of the cells, and a flattening of the cell tips (figs. 4, pl. 6; 11, pl. 7).

At the time of the tearing and breaking, varied origins of the cavities are noted. In some cases the tissue is composed entirely of the fundamental undifferentiated hyphae of the homogeneous basidiocarp, and the cavity primordia can only be noticed by the loose arrangement of the hyphae. Other cavity primordia showed several knots of differentiated tissue scattered near the opening (fig. 3, pl. 6), while sometimes these palisade primordia had outlined the cavity, but in a most irregular tangled manner. A third type had the palisade in some cases well formed at the time of splitting (fig. 5, pl. 6). The palisade was either lining only a part of the cavity and was progressing with the splitting (fig. 13, pl. 7), or it was completely formed, outlining the shape of the future cavity, and the opening came later (fig. 5, pl. 6).

From the different types it is noticed that the sequence of order of the splitting, differentiation of tissue, and final formation of the palisade is varied, depending on the age and the conditions under which the basidiocarp is developing. Variations in origin and all stages of development may be found in the same fruiting body.

Study of the subsequent development and differentiation of the fruiting body confirmed in general the description given by Rehsteiner (1892). The first cavities to arise are the fertile ones followed closely by the sterile cavities in the basal part as shown in figures 22 and 23 of plate 8. The sterile cavities arise in the same manner as the fertile ones from the fundamental " building " tissue. These are distinguished from the fertile cavities by their regular, spherical shape and the lack of progressive enlargement.

Now the fertile cavities develop progressively toward the periphery from the dome-shaped region of the undifferentiated tissue surrounding the first glebal region. These likewise originate in the same manner as the first cavities. With the expansion of the fruiting body and increase for a time of the fundamental tissue between the cavities, new cavities arise in the midst of those formed earlier. Very rapidly the formation of both sterile and fertile cavities and the differentiation proceed until a form with many cavities is reached. From this time on no new elements arise, but an enlargement of those already present takes place.

The sterile cavities increase somewhat in size but remain spherical, regular in shape, and separated by a large amount of fundamental tissue. The fertile cavities, on the other hand, progressively increase in size and become elongated

and lobed.   Several cavities merge together so that the cavity becomes much longer in proportion to its width.   The cavities tend to assume a radial direction, the long axis directed toward the periphery (figs. 24, 25, pl. 8).   They are arranged close together with a small amount of fundamental tissue between them (fig. 26, pl. 8).   Thus the regular, round, sterile cavities scattered in the fundamental tissue in the basal part of the fruiting body and the irregular, elongated, labyrinthine, radially arranged, fertile cavities filling the greater part of the basidiocarp and placed close together can easily be distinguished.

The palisade-like tissue already described as surrounding the cavities becomes the tramal plates.   Surrounding the sterile cavities the palisade tissue differentiates no further but remains a firm, compact layer made up of hyphae with three or four short cells, the lateral walls being closely appressed and crowded against each other and with the tips flattened (fig. 11, pl. 7).   On the other hand, the palisade tissue around the fertile cavities continues to increase and the cells continue to divide.   Because of the greater expanse in the top part of the basidiocarp, hyphae of the tramal plates are not so firmly compact but are in a looser palisade arrangement.   The palisade hyphae branch, and characteristically each one forms two basidia at the tip (figs. 12, 14, pl. 7).   The tip cells pointed toward the cavity are elongated and become swollen, with the ends rounded to form the basidia (fig. 14, pl. 7).   The fundamental tissue between the cavities disintegrates and dissolves away, leaving the sterile cavities surrounded by the palisade-like tissue and the fertile cavities bordered by tramal plates and basidia.

Wherever the nuclei were distinguishable, a binucleate condition was found. The "cortex," "building," and smaller differentiating "building" hyphae of the rhizomorph have two nuclei in each cell (figs. 1, pl. 6; 16, pl. 7).   In the larger differentiated "building" hyphae and "conducting" hyphae the nuclei were not seen.   The binucleate cells are present in all hyphae throughout the development of the basidiocarp: in the fundamental tissue, cells of the various zones of the peridium, early palisade, subhymenial layer, and basidia (figs. 3, pl. 6; 11, 12, 13, 17, pl. 7).   The nuclei are minute and small in proportion to the size of the cell, but in the outer peridium cells where detailed study was made the nuclei are slightly larger (fig. 17, pl. 7).

Hemispherical pads were associated with the cross walls of all the cells of the fruiting body and of the rhizomorph with the exception of the "fiber" hyphae (figs. 1, 3, 4, pl. 6; 12, 14, 16, pl. 7).

SUMMARY

The young rhizomorph consists of three regions: central, fibrous, and cortex.   The cells are typical for each region.

The presence of hemispherical pads on the septa of all the cells with the exception of the "fibrous" hyphae is noted.

The basidiocarp arises from the homogeneous mass of undifferentiated hyphae.   It may also proliferate out of older forms, arising from the undifferentiated tissue inside the peridium.

The first differentiation is the radial arrangement and the swelling of the peripheral cells for the formation of the exoperidium.   The first indication of the glebal cavities is the presence of darker-staining hyphal tips arranged in irregular knots.   Such hyphae become palisade tissue surrounding the cavities and later form the tramal plate with basidia at the tips of those around the fertile cavities.

The opening of the cavity arises by the expansion, stretching, and splitting of the fundamental hyphae.   Glebal cavity primordia vary in their origin and different stages of development of the surrounding palisade at the time of the splitting.

The fundamental hyphae disintegrate, and the cavity opens by means of stretching, tearing, plasmolyzing, swelling, and gelatinization.   Progressively the fertile cavities enlarge and change in shape.

The zones of the peridium differentiate very early in the development of the basidiocarp, and this differentiation is correlated with the stages of development of the glebal cavities and the size of the fruiting bodies.

The morphology of the successive representative stages of the development of the basidiocarp until formation of the basidia is described.

All cells of the fruiting body and of the rhizomorph, with the exception of the " fiber " and " conducting," where no nuclei were seen, are binucleate.

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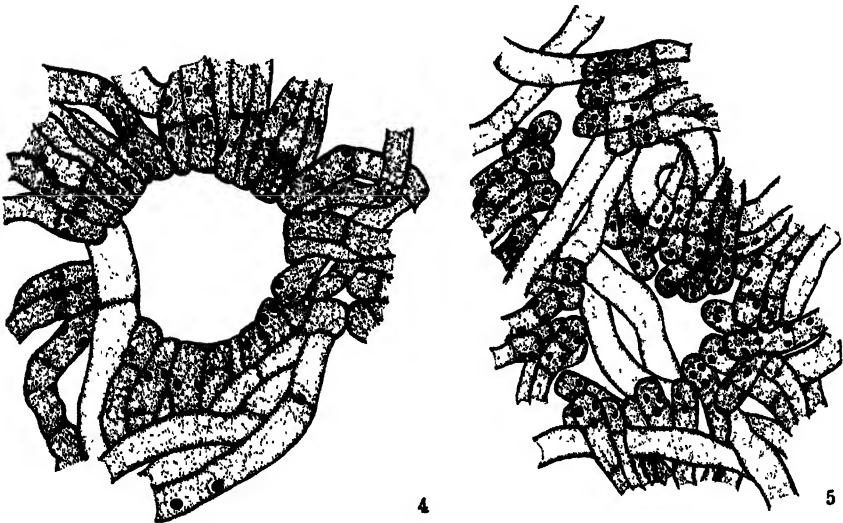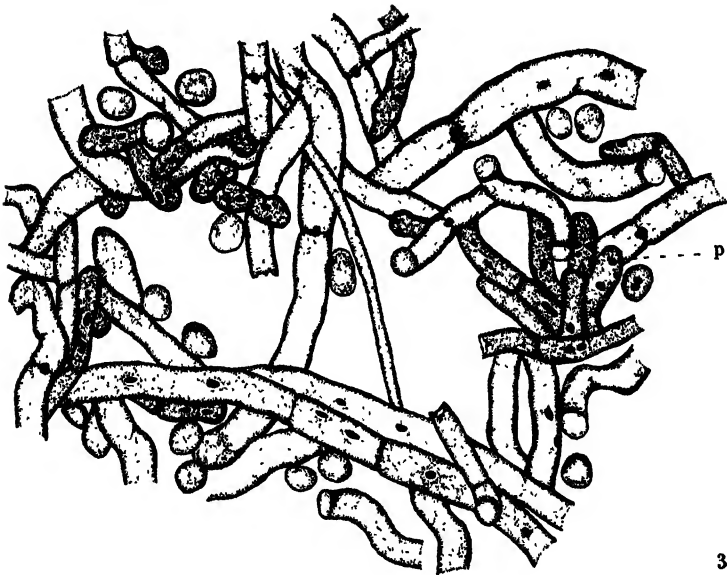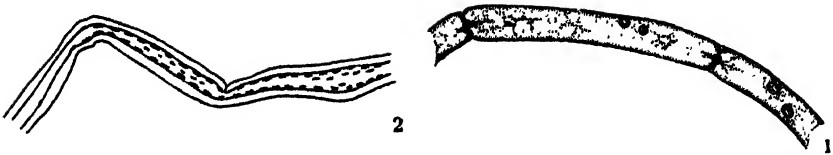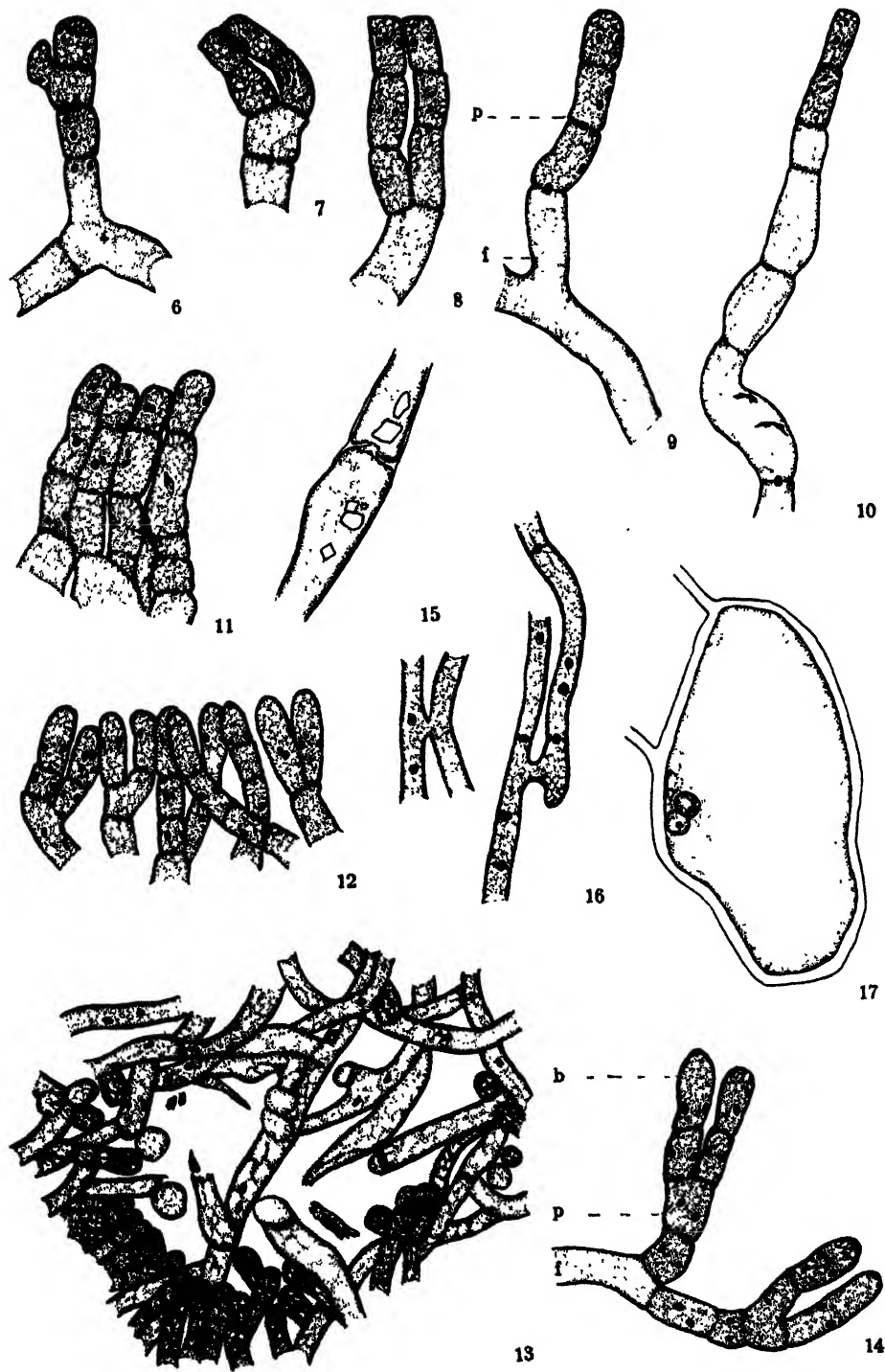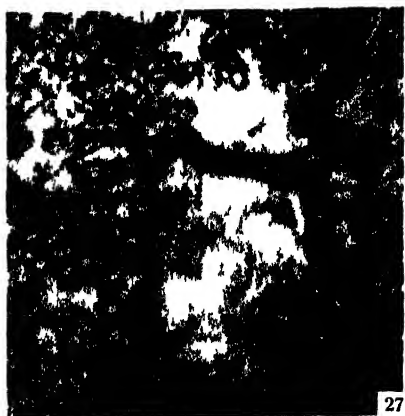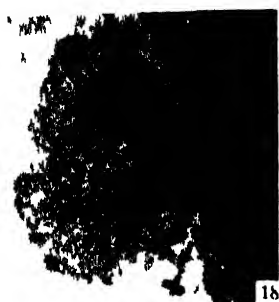
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EXPLANATION OF PLATES

All drawings were made with the aid of a Spencer camera lucida and drawn with the Spencer achromatic objective 1.5 mm. Magnification of figures 1–5, 13, 15, 16, about × 1840; figures 6–12, 14, about × 2300.

PLATE 6

FIG. 1. Portion of typical "building" hyphae with binucleate cells and protoplasmic connections.

FIG. 2. Type of "fiber" hyphae with thick walls and rows of granules.

FIG. 3. Type of origin of cavity in which splitting and stretching preceded palisade differentiation. Early differentiation of cells shown at *p*.

FIG. 4. Section through small diameter of enlarged sterile cavity showing even contour of palisade tissue.

FIG. 5. Type of origin of cavity in which palisade is formed before splitting.

PLATE 7

FIG. 6. Origin of branching of the palisade cells.

FIG. 7. Stage in the branching and formation of palisade tissue.

FIG. 8. Cell of fundamental hypha branched and forming two palisade hyphae.

FIG. 9. Fundamental hypha (*f*) differentiated to form palisade tissue (*p*).

FIG. 10. Fundamental hypha with differentiating palisade which pushed in after palisade was fairly well formed.

FIG. 11. Portion of palisade tissue around sterile cavity.

FIG. 12. Portion of hymenial layer with basidia at tips surrounding the fertile cavity.

FIG. 13. Stage in development of cavity showing palisade formation and disintegration of fundamental tissue.

FIG. 14. Fundamental hypha (*f*) with palisade tissue (*p*) and basidia (*b*) at tip.

FIG. 15. Differentiated "building" hypha with broken crosswall and prominent crystalloids.

FIG. 16. Anastomosing of typical "cortex" hyphae with binucleate cells and protoplasmic connections.

FIG. 17. Cell of exoperidium of basidiocarp with two nuclei.

PLATE 8

FIG. 18. Primordium of basidiocarp showing connection with a rhizomorph at base of older form. × 90.

FIG. 19. Young basidiocarp with definite peridium region and a few glebal cavity primordia. × 35.

FIG. 20. Primordium of basidiocarp connected with inner undifferentiated region of older fruiting body. × 90.

FIG. 21. Basidiocarp showing inner and outer peridium and glebal region. × 25.

FIGS. 22, 23, 24. Older basidiocarps with glebal cavities increasing in size and number. × 25.

FIG. 25. Portion of mature basidiocarp with fertile cavities lined with basidia. × 25.

FIG. 26. Portion of glebal region of basidiocarp with developing cavities. × 350.

FIG. 27. Developing cavity with palisade (*p*) at lower side and fundamental tissue (*f*) disintegrating. × 1100.

21

MORPHOLOGY OF THE MEGAGAMETOPHYTE AND THE EMBRYO SPOROPHYTE OF *ISOETES LITHOPHILA*

CHARLES LA MOTTE

Isoetes has long been the subject of investigation and discussion, but Hofmeister (10), as a result of his investigations on *Isoetes lacustris*, gave us in 1862 the first detailed account of the life history of the genus; and in most particulars his interpretations have stood as correct up to the present time. He called special attention to the similarities existing between *Isoetes* and the Lycopods, and pointed out some of the homologies existing between *Isoetes* and certain Spermatophytes, especially the Conifers.

Later morphological studies on *Isoetes* have been made by Bruchmann (4), Belajeff (2), Kienitz-Gerloff (11), Farmer (8), Campbell (5), Arnoldi (1), Goebel (9), Scott and Hill (14), Smith (15), Stokey (16), West and Takeda (17), and, in a limited way, by others. But accounts of detailed studies of the female gametophyte and the embryo sporophyte are pretty well limited to the work of Hofmeister (10), Kienitz-Gerloff (11), Farmer (8), Campbell (5), and Arnoldi (1). Hofmeister investigated *I. lacustris,* a species found in the British Isles and in North and Central Europe. This species, according to Pfeiffer (13), has megaspores 500–700 microns in diameter. Kienitz-Gerloff also used *I. lacustris* in his study of the embryo. Campbell worked on *I. echinospora* var. *braunii* (*I. braunii*), whose megaspores measure from 420 to 580 microns in diameter. Farmer studied material of *I. lacustris*, but most of his account deals with the development of the organs of the mature sporophyte. Arnoldi made a study of the female gametophyte of *I. malinverniana.* This form is limited in its distribution to Piedmont, in Italy, and has large megaspores ranging from 660 to 880 microns in diameter. *Isoetes lithophila* Pfeiffer, used in the present investigation, is apparently limited in its distribution to a small area in Central Texas, and has very small megaspores ranging in size from 290 to 360 microns.

The mature gametophyte of this species exhibits a striking abundance of rhizoids after the first leaf of the embryo has appeared, as well as a conspicuous protrusion of gametophytic tissue, which for a time encloses the first leaf of the embryo and later remains as a sheath about the base of the leaf until the gametophyte disintegrates. Observation of these prominent features led to the present investigation, and as it progressed, several interesting morpho-

logical characteristics of the young embryo presented themselves, chief of which is the shifting orientation of the embryo during its early stages of development. This point, especially, should prove of value in determining the affinities of the Isoetaceae.

MATERIALS AND METHODS

The first *Isoetes lithophila* material used in this work was collected on November 11, 1928. A rain had fallen during the preceding week, and the depressions in which the plants were growing were well filled with water. Small leaves had sprung up to a height of 2–3 cm. above the soil. Numerous megaspores were found in and on top of the soil and gravel. Portions of the earth containing corms, megaspores, and small plants were scooped up and brought into the laboratory, where they were placed in open chambers and kept well watered. Distilled water was used for the first month or two, but tap water soon proved to be equally as good. About three weeks later many megaspore coats were splitting along the ventral ridges, and on December 5 a number of green embryo leaf tips were observed protruding from the gametophytes. On December 11, just one month after collecting the material, rather large numbers of young sporophytes were making their appearance.

Another trip was made to the mountains at Christmas time, and more material brought in. Neither the old sporophytes nor the gametophytes were as far advanced in growth and development as those previously brought in and kept in the laboratory. Very few mature prothallia could be found, and scarcely any embryos' were in evidence. Within a few days, however, germinating megaspores became more numerous; but germination was so far from simultaneous that many of the spores did not open until one month later. I was able, therefore, to select embryonic material from day to day over a considerable period.

During the summer of 1930, plants having mature sporangia were collected and the spores were removed for planting, but germination results were disappointing. The following year another collection was secured, and the spores were planted under a variety of conditions. Some were planted on soil brought in with the plants and watered with pure rain water in an attempt to duplicate the conditions of their native habitat. Under no conditions, however, did the germination exceed ten per cent, the spores from many sporangia showing no viability whatever.

This low percentage of germination together with lack of simultaneous germination necessitated the tedious task of picking out the mature gametophytes one at a time for killing and embedding.

Two or three lots of megaspores were killed and fixed in formalin-acetic-alcohol (formalin, 5 per cent; acetic acid, 2½ per cent; alcohol, 45 per cent; water, 47½ per cent). They were then run up to absolute alcohol, cleared and infiltrated with xylol, and embedded in 56-degree paraffin. The remain-

ing lots were killed and fixed in weak chromo-acetic solution (chromic acid, 0.7 g.; acetic acid, 1 cc.; water, 100 cc.). They were then run up in diffusion shells through absolute alcohol into chloroform and embedded in paraffin. Due to the very small size of the megagametophytes, no attempt was made to orient them for sectioning in a given plane.

The paraffin-embedded material was sectioned to thicknesses of 4–10 microns; most of it was cut at 5 microns. All staining was done after the sections were fixed on the slides and the paraffin was removed. Three stain combinations were used, and each combination was at least partially success-ful. First, safranin-gentian violet was used by the method of overstaining and removing the surplus with alcohol. Next, Flemming's triple stain was used, and results in many instances were very satisfactory. Last, Heiden-hain's iron-alum haematoxylin was used, followed by gold-orange (Orange II) dissolved in clove oil as a counter-stain. This gave some very satisfactory preparations with good definition.

<div align="center">OBSERVATIONS</div>

<div align="center">*The female gametophyte*</div>

The structure and contents of the megaspore of *Isoetes lithophila* seem to be similar to those described by the various investigators for other species. The spore wall appears to be made up of at least three distinct layers, the outer one of which is much thicker than either of the others and is composed of a hard, brittle material. The spore is conspicuously filled with starch grains and other reserve foods, most of which color deeply with the stains used in this work.

The primary nucleus was frequently observed, and every time its location could be ascertained with any degree of certainty, it proved to be in the basal portion of the spore (text fig. 1). This corresponds to the location of the primary nucleus in *I. echinospora* (5), but in *I. lacustris* it is located in the apex of the spore (8), and this is also the case in *I. malinverniana* (1).

The early stages of megaspore germination up to the time of first tissue formation were not observed in any of my preparations, but they are probably similar to the corresponding stages observed and described by Campbell (5). He found that the primary nucleus first divides in its original position. A cell-plate becomes evident between the two daughter nuclei, but no wall is formed. Prior to or just following the next division, these two secondary nuclei or their four daughter nuclei move up to the apex of the spore. There, free nuclear division continues until 30 to 50 nuclei are formed. Then divi-sion walls appear simultaneously, and cell formation takes place just beneath the apex of the megaspore.

Among my preparations were sections of a few gametophytes which had apparently just reached this stage. There were only two or three layers of tissue beneath the apex of the spore coat, the cell walls were thin, there was
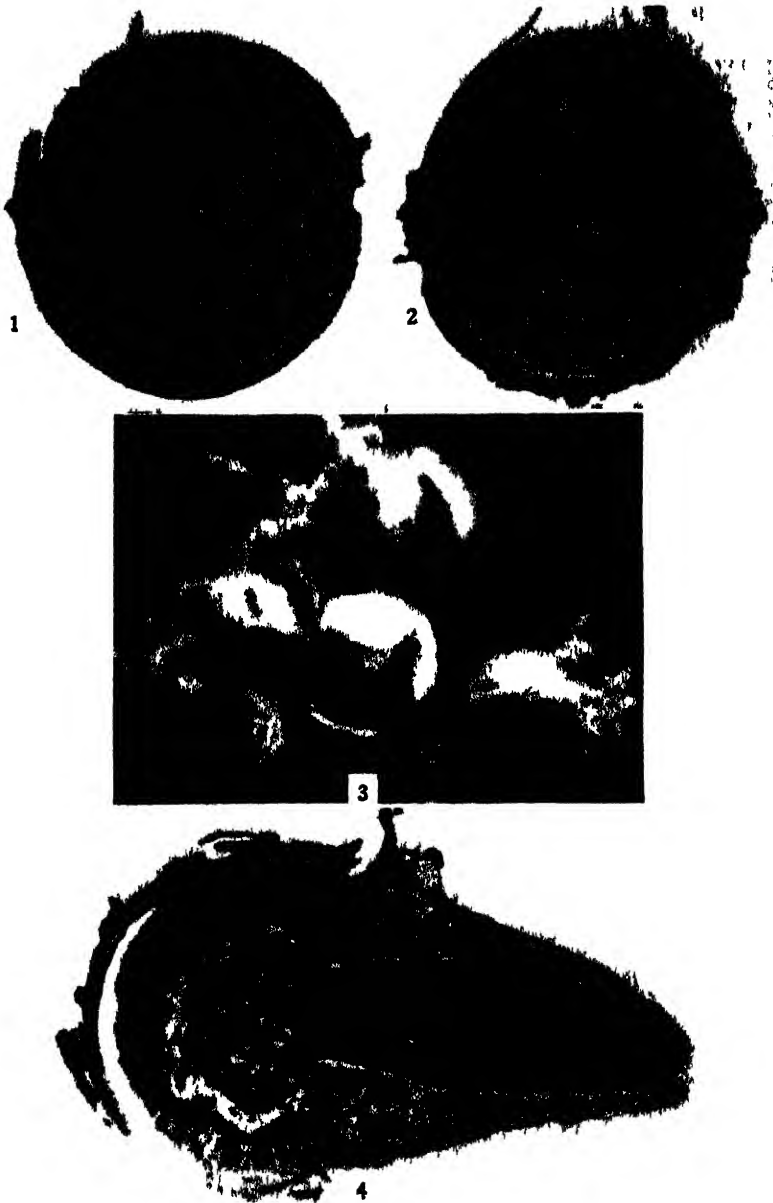
Text Figs. 1–4. Fig. 1, section through megaspore, with apex at the top and nucleus in basal region; epispore removed. × 180. Fig. 2, approximately median vertical section of megagametophyte showing first archegonium, containing few-celled embryo sectioned tangentially. Fig. 3, megagametophytes bearing numerous rhizoids made especially conspicuous by clinging soil particles. × 44. Fig. 4, transverse section through apical region of prothallium and advanced embryo, showing the large calyptra enclosing the primary leaf. × 180.

no evidence to indicate that the megaspore was cracked, and no archegonia had begun to form.

From this early stage, cell formation proceeds towards the base of the spore, chiefly following the spore wall. It is evidently rapid at first, until the upper portion of the spore is well filled with fairly thick-walled cells; but cell formation in the base of the spore is very slow, and possibly is very often not complete until after the embryo is well advanced. Some of my best slides fail to show cell walls in the bases of spores having embryos fully half-developed to the point of protruding from the gametophyte. Others do show tissues throughout the gametophyte at this stage and even earlier with reference to the embryo. At any rate, the cells in the lower part of the
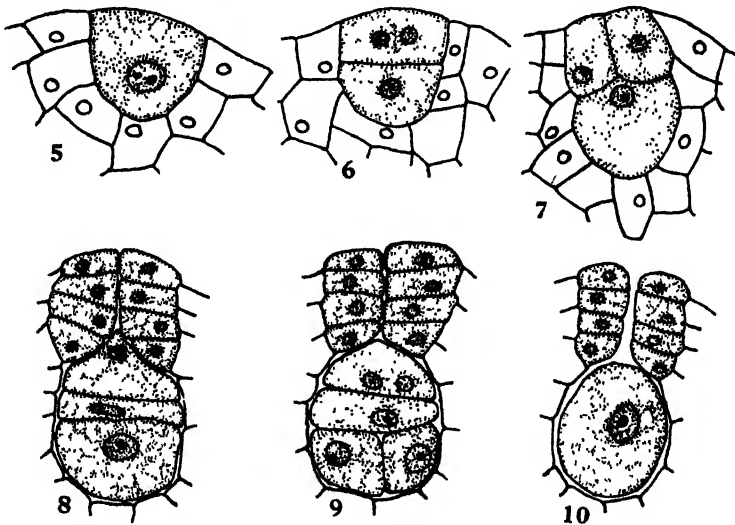
Text Figs. 5–10. Figs. 5-7, successive stages in early development of the arche-gonium. Fig. 8, median section through complete archegonium composed, from the apex downward, of neck cells, neck-canal cell, ventral-canal cell, and the immature egg cell. Fig. 9, an unusual archegonium with two neck-canal nuclei and two egg cells. Fig. 10, mature archegonium after neck-canal and ventral-canal cells have disintegrated. × 450.

prothallium are much larger when present than those of the portion near the apex, and the walls are very thin. Starch grains and other food particles are prominent in the basal tissue but not in the apical. The change from the apical to the basal type of tissue is often quite abrupt in this species, but there is no evidence at any stage of a diaphragm separating the two portions of the gametophyte (text figs. 2, 16).

The development of the archegonium in this species does not differ from that described by other investigators. The first archegonium initial appears early in the prothallial development, at or very near the apex. It is much larger than the cells surrounding it; and according to Campbell (5), " it is simply one of the first-formed cells that ceases to divide after it is complete; and as the neighboring cells divide rapidly, the contrast in size between it and

those adjoining becomes very marked." The nucleus becomes large and the contents become dense (text fig. 5). Then division occurs in a transverse plane giving rise to an outer cell, which through successive divisions forms the neck, composed usually of four tiers of four cells each, and an inner one, which gives rise to the neck-canal cell and the central cell. The central cell then divides once more to form the ventral-canal cell and the egg cell. By the time this is accomplished the neck cells and the venter of the archegonium are usually complete (text figs. 6, 8). The cells of the venter below the neck cells do not have their origin in the archegonium initial but in the surrounding tissue, and these cells are somewhat smaller than others of the prothallium. The nucleus of the neck cell may divide once more (text fig. 9), and according to Lyon (12) a wall may form between the two daughter nuclei, but such wall formation was not observed in this species. In rare instances the cell that normally enlarges and rounds off to become the egg divides to form two before the neck-canal and the ventral-canal cells disintegrate (text fig. 9). Two fully mature eggs in one archegonium were not observed, however, nor were two embryos seen which might have originated in the same archegonium. Such instances were described by Lyon (12) for *Selaginella* and might reasonably occur in *Isoetes*. Be that as it may, the neck-canal and the ventral-canal cells very soon disintegrate as the egg enlarges and rounds off, and the neck of the archegonium opens up for the entrance of spermatozoids (text fig. 10).

Very shortly after the archegonium is mature, and whether the egg is fertilized or not, the two upper cells of each tier of the archegonial neck begin to disintegrate, leaving a dark brown spot which marks the presence of the old archegonium (text fig. 3). At least one or two more archegonia ripen immediately after the first, and if fertilization does not occur in any of these, other archegonia are formed continuously throughout the life of the prothallium or until fertilization occurs. Fully a dozen or more in various stages up to the point of disintegration may quite often be seen in a single gametophyte, and new archegonia keep appearing until the prothallium becomes almost transparent and finally dies as a result of having exhausted its food supply.

The process of fertilization was not observed, nor has it been reported for *Isoetes* by other investigators. Two embryos in separate archegonia are sometimes found in a gametophyte, one few-celled and the other quite advanced. It is possible, perhaps, that two embryos may develop simultaneously, but this was not observed in my preparations.

Rhizoids

The prominence of rhizoids on the mature gametophyte is a striking feature. They are made especially conspicuous by the proportionately large amount of soil and dead organic particles normally adhering to them, and they are so firmly attached to many such particles that rather prolonged washing in running water will not loosen them (text fig. 3).

The rhizoids have their origin in the superficial cells of the gametophyte on the surface exposed by splitting of the spore coat. They usually appear first in the immediate vicinity of an archegonium whose top tiers of neck cells are breaking down. Later, they may develop in extremities of the triradiate crack, thus forming from one to three rather uniform tufts. When one of the superficial cells of the prothallium first shows evidence of being a rhizoid initial, its protoplasm is more dense, the cell as well as the nucleus is larger, and the exposed surface is rounded out beyond the surface of surrounding cells (text fig. 11). A tubular protrusion then develops, and soon
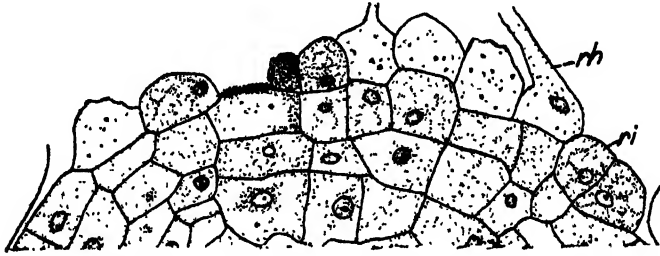
Text Fig. 11.   Apical tissue of an old prothallium bearing many rhizoids (*rh*) and rhizoid initials (*ri*). × 450.

the nucleus begins to move out into it. I have seen no evidence that septations occur in the rhizoids, nor that the nucleus ever divides after the rhizoid initial is formed. These rhizoids may become a millimeter or more in length and persist, apparently, throughout the life of the gametophyte.

From few to many rhizoids almost invariably precede the embryo in making their appearance; and embedded and sectioned material revealed the fact that rhizoids may occasionally be present when there is no embryo developing within the gametophyte, but this is probably rare. Whether all exposed cells of the prothallium are potential archegonium and rhizoid initials is uncertain; but it is true that in some areas of the gametophyte every superficial cell, except the archegonial neck cells, is rhizoidal in character (text fig. 11).

The calyptra

If, in the gametophyte, an embryo is advancing in its development so that the leaf protrudes beyond the place representing the original periphery of the prothallium, the protruding part is invariably enclosed for a time by a sheath of tissue, which I shall call " calyptra " (text fig. 4). The use of this term as applied here to the gametophytic sheath cannot, probably, be entirely justified, because the archegonium is not involved in the formation of this tissue and neither is it a part of the tissue as it usually exists. " Pseudocalyptra " might be used, but for the sake of simplicity in terminology I shall use " calyptra " in the modified sense just mentioned.

The calyptra of *Isoetes* is not merely the result of mechanical stretching

of gametophytic tissue up to the rupturing point. Cell multiplication takes place in the two or three outer cell layers of the prothallium just ahead of the embryo leaf-tip before there is any evidence of bulging. Few mitotic spindles were seen in the preparations demonstrating this feature; but since these figures were rather small and inconspicuous, many others could have
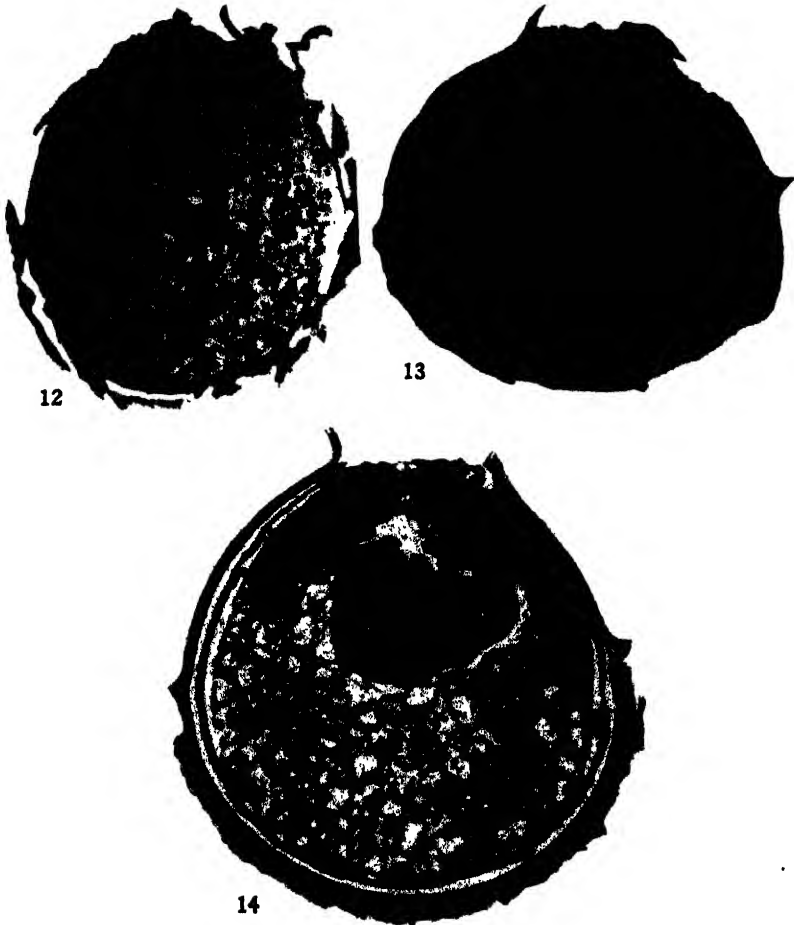

Text Figs. 12–14. Vertical sections through megagametophytes and young embryos. Figs. 12 and 13, before the embryo shifts. Fig. 14, after the embryo has shifted into its final position with reference to the apex of the prothallium. The archegonial neck cells may be seen above each embryo. × 180 and 200.

been overlooked, especially where the stains were not at their best. However, other evidence of cell division is quite beyond question. Finally, the leaf breaks through the tissue, so that no cap remains on the leaf tip; and this structure remains for some time as a gametophytic sheath around the base of the leaf, eventually being torn apart by the advancement of the second leaf and the enlargement of the embryo in general.

Very little of such tissue is formed over the protruding primary root, which merely breaks through the periphery of the gametophyte without causing much bulging at that point.

The embryo

The first division of the fertilized egg of *I. lithophila* was observed in metaphase with the poles of the spindle almost in line with the axis of the archegonium. Two-celled stages were not seen with any degree of certainty, but judging from this mitotic figure it is very probable that the first wall formed is transverse "but more or less inclined to the axis of the archegonium," as reported by Campbell (5) for *I. echinospora*.
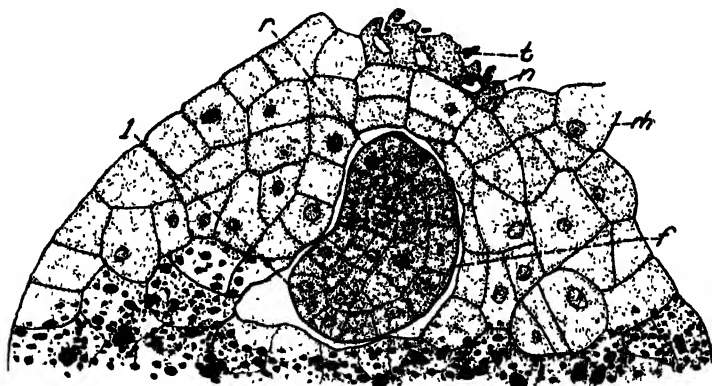
Text Fig. 15. Section through a mature prothallium, showing details of the apical tissue and position and orientation of early embryo; disintegrating neck cells, *t*; normal neck cells, *n*; rhizoid, *rh*; primary leaf of the embryo, *l*; root, *r*; foot, *f*. × 400.

Though the nuclei were often sufficiently distinct, I could not clearly distinguish cell walls in any of the youngest embryos examined. They could be traced very well, however, after the embryo had become many-celled and was elongating appreciably.

At first, the few-celled embryo is more or less globular and occupies the space within the archegonial venter previously occupied by the egg (text fig. 12). Then the rather thin-walled cells making up the base of the venter are gradually digested by the young embryo, which at the same time begins to elongate. This continues until the embryo is noticeably longer than broad, with one end close up to the neck of the archegonium and the other extending toward the center of the prothallium. Hence the direction of greatest growth of the embryo at this early stage is in the direction away from the neck and in a line corresponding roughly to the axis of the archegonium (text fig. 13).

After some elongation, growth is more rapid on one side of the embryo than on the other, thus bending the lower end slightly to one side. The direction of most rapid growth is now changed, shifting some 30 to 40 degrees from the original axis of elongation. Growth is occurring simultaneously,

however, in every dimension of the embryo so that it takes on something of the shape of a distorted ovoid structure, straightened or slightly concave on one side and bulged on the opposite side (text fig. 15). The initial cell of the ligule soon becomes clearly distinguishable, and at the same time the end of the embryo farthest from the neck of the archegonium becomes more pointed. This portion may then be recognized, by virtue of its longer, pointed end and its corresponding position in later stages, as the first leaf. Subsequent enlargement makes the end nearest the canal of the archegonium too large to be contained in the remaining bell-shaped portion of the venter. Also, the convex portion of the embryo, which later becomes the foot, begins simul-
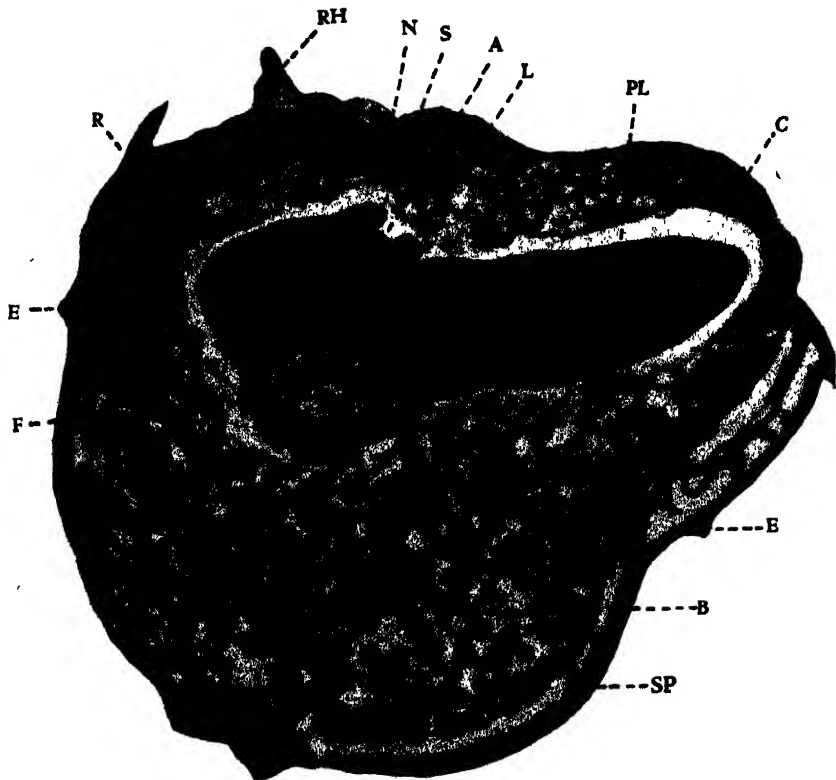
Text Fig. 16. Median section through megagametophyte and advanced embryo; rhizoid, *RH*; archegonial neck, *N*; calyptra, *C*; spore coat with outer brittle layer removed, *SP*; equatorial ridge, *E*; root of embryo, *R*; leaf sheath, *S*; stem apex, *A*; ligule, *L*; primary leaf, *PL*; foot, *F*. × 300. (Photographed by J. T. Buchholz.)

taneously to digest more actively the gametophytic tissue below. These two phenomena combined—that is, the enlargement near the old archegonial neck plus digestion of the gametophyte by the foot—cause the embryo to shift into a tangential position with its long axis almost parallel to the apical surface of the prothallium, instead of lying almost perpendicular to it as at first.

Even at the time of shifting into this final position the embryo is very small. The ligule may have advanced only to the one- or two-celled stage; the region which is to give rise to the root is scarcely recognizable, except by reference to the position of the ligule and foot; and the leaf is just becoming appreciably extended. The photograph (text fig. 14) of a stage somewhat later than that just described gives an idea of the size of the embryo compared with that of the gametophyte in which it is embedded.

From this time on, the embryo enlarges rapidly without much, if any, further shifting in its position relative to the axis of the archegonium. The leaf grows on toward the periphery of the prothallium, and just ahead of it cell multiplication takes place in the gametophytic tissue to form the calyptra. The root begins to take on a definite form and extends itself in the opposite direction from that taken by the primary leaf. A sheath develops at the base of the leaf and extends around each way to the base of the root. This sheath encircles the ligule together with some of the cells below it, cells that later give rise to the second leaf and the stem apex. The ligule grows continually by cell divisions in both transverse and longitudinal planes until it forms a flat appendage several cells long by about as many broad before the first leaf emerges from the calyptra. The foot continues to enlarge into the nutritive tissue of the gametophyte, absorbing the stored food for the nourishment of the embryo (text fig. 16).

All of this growth proceeds rapidly; for within a week or ten days after the megaspore cracks open, a young sporophyte may be seen emerging. The green apex of the primary leaf makes its appearance first and may be recognized while it is still enclosed within the calyptra. Shortly afterward, when the primary leaf has reached a length of 4–6 mm., the first root breaks through the epidermal tissue of the gametophyte. Then in a week or two a second leaf appears and soon a second root. In the meantime root hairs develop on the first root after it becomes 1–2 mm. in length, and the young sporophyte is now ready for an independent existence; but it may continue to draw upon the gametophyte for some time before the food materials of the latter are entirely exhausted.

DISCUSSION

Observations made in this study on the female gametophyte of *I. lithophila* agree essentially with Campbell's observations on the gametophyte of *I. echinospora*. His account of the early germination stages is much more detailed and complete, however, than my observations have permitted; but with regard to the mature gametophyte interesting features have been noted which were not fully treated in his papers nor in those of other investigators.

Since the location of the primary megaspore nucleus seems to vary among different species, it is probable that the location of this nucleus with reference to the apex of the spore is of little morphological or physiological importance. The congregation of free nuclei in the apex before tissue formation begins is

the thing essentially worthy of notice, but I was not fortunate enough to observe this phenomenon in any of my preparations.

The more advanced prothallium of *I. lithophila* is composed of two distinct types of tissue: the firmer " reproductive " tissue of the apical region and the looser " vegetative " tissue of the basal region abundantly filled with starch and other food materials.   Change from the apical to the basal type of tissue is usually quite abrupt but by no means regular and definite enough to suggest the presence of a diaphragmatic membrane such as that described by Campbell (6) in *Selaginella kraussinana*.   The region in which these two types of tissue merge lies just beneath the archegonial venters.   Consequently, the embryo has easy access to the more highly nutritive portion of the prothallium immediately after digesting its way through the basal cells of the venter.

Development of the archegonium of *I. lithophila* seems to be identical with that described for *I. lacustris* (10) and *I. echinospora* (5).   If fertilization is prevented, many archegonia will form before the prothallium disintegrates.   It is very probable that the number which may develop in a single gametophyte is not definite and depends solely upon the abundance of food originally stored in the megaspore.   This agrees with Farmer's (8) opinion, but differs from that of Campbell (5), since Campbell believes that the number is definite and depends upon the number of potential initials laid down as the apical tissue of the gametophyte is formed.   In the species studied by him, however, he found that only a small number, " probably never more than five or six," in addition to the first three are ever formed; but in *I. lacustris* Kienitz-Gerloff (11) observed that there may be 20 to 30 formed, and in both *I. lithophila* and *I. melanopoda* I have seen a large number on some old prothallia.   Hofmeister (10) states that gametophytes of *I. lacustris* may live six months or longer and still bring forth archegonia that are apparently normal in every way.   Hence, in view of the small maximum number of archegonia found by Campbell, his view is reasonable; but in view of the much larger number observed by others as well as myself, it seems equally as reasonable to conclude that the number is not definite.

Apparently, neither the rhizoids nor the calyptra have received much attention from anyone up to the present time.   Campbell (5, 6) merely makes mention of the rhizoids by saying that " only in the rarest instances were root hairs developed from the superficial cells," and he recognizes the presence of the gametophytic sheath in the following sentence (6) : " The embryo is for a long time covered by prothallial tissue, which in the upper part continues to grow with it; but finally cotyledon and root break through, the former bending upward, the root bending down and anchoring the young sporophyte in the mud."   Arnoldi (1) mentions the rhizoids of *I. malinverniana* and shows a few of them in his figure 10.

It is possible that neither the rhizoids nor the calyptra have any great physiological or taxonomic importance, but each at least helps to give an external appearance to the prothallium that makes it closely resemble that of *Selaginella*.

Then, too, it is worthy of note that the rhizoids and calyptra taken together add considerably to the amount of gametophytic tissue that is not confined within the spore walls.

After a careful consideration of my observations on the embryo and after a careful study of the drawings of Hofmeister (10), Kienitz-Gerloff (11), and Campbell (5)—all essentially agreeing—I cannot fully understand every aspect of Campbell's interpretations.   As previously pointed out, it is evident that the embryo begins its development by radial elongation and then shifts into a tangential position so that it comes to lie more or less parallel to the apical surface of the prothallium rather than roughly perpendicular to it, as
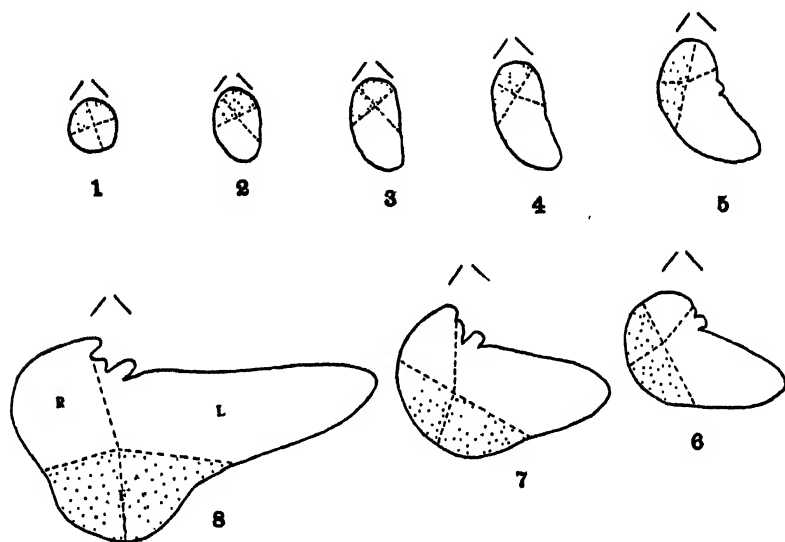
Text Fig. 17. Series of diagrams to illustrate shifting orientation of the embryo. The lines above each represent the apex of the archegonial venter; root, *R*; primary leaf, *L*; foot, *F*; regions of embryos (represented by broken lines and dotted areas) are largely hypothetical.

at first.   Campbell makes no mention of this shifting orientation, but on the other hand he indicates that the elongation of the few-celled globular embryo is in the same plane and direction as the axis of the advanced embryo. On this basis he interprets the two upper quadrants of the few-celled embryo as giving rise to leaf and root, respectively, while the lower quadrants together develop into the foot. Unfortunately, I was unable to obtain mounts demonstrating unmistakably the quadrant or octant divisions of the embryo; so my interpretation of the portions giving rise respectively to leaf, root, and foot is based entirely upon relative shapes and positions of parts traced back from the advanced embryo to younger stages.

On this basis, however, it appears that the lower portion of the few-celled embryo gives rise to the leaf, and one of the portions nearest the archegonial neck cells gives rise to the root, while the remaining portion contributes to

the foot; and it is possible, even, that the two upper "quadrants" give rise to the foot, while the two lower "quadrants" give rise to the leaf and root, respectively. This latter possibility is illustrated by the broken lines and shaded areas in the following series of diagrams (text fig. 17). The outlines and orientations with reference to the apex of the archegonium were made from actual observation.

Regarding the shapes and forms of the embryos in various stages, my observations do not materially disagree with Campbell's figures (5, pl. 16); and assuming that the neck of the archegonium is above the embryo in his figures 26a and 26b (as is indicated by the arrow in his figures 20, 21, 24), there is also agreement regarding orientation up to this stage. I failed, however, to see an embryo at such an early stage oriented as is indicated by the arrow in figure 27 of Campbell's drawings. Had the arrow been placed at or near one end on the upper side and pointed directly away from the approximate center of the embryo, it would again have been in exact accordance with my observations. The orientation indicated in his figure 28 is almost in agreement with that of similar embryos observed by me. Judging from my material, however, this would be more typical if the arrow were shifted some 25 or 30 degrees toward the ligule. It seems, too, that the "r" and "l," designating root and leaf, respectively, should be interchanged. All later stages shown and described by Campbell are just as I have found them in *I. lithophila*, except that I have never observed a primary leaf growing up through the neck of the archegonium.

Hofmeister (10) noticed that the "rudiment of the embryo" grows toward the mid-point of the spherical prothallium by repeated division of the cells turned away from the neck of the archegonium. His figures demonstrating this stage (pl. 46, figs. 22, 23) seem to be correct in regard to the shape and position of the embryo, but it is possible he did not have evidence enough to justify labeling the end nearest the neck of the archegonium the leaf, "fr^1." It is likely that the portion at the opposite end labeled "ax" should be designated as the leaf. At any rate, shapes and forms of all stages seem to be well shown in his figures (pl. 46, figs. 22, 23, 24; pl. 47, figs. 1, 2). For our present purpose, however, it is to be regretted that he did not designate the position of the archegonium in each case. Had he done so, doubt as to orientation would have been eliminated. He also reports observing the primary leaf growing out through the neck of its "mother" archegonium or sometimes growing in the opposite direction through the center of the prothallium. Neither condition was observed in any of my material.

The figures of Kienitz-Gerloff (11, pl. 7, figs. 8A–14, incl., and 28) show clearly the forms assumed by the embryo in early stages, but the orientation is not definitely pointed out by him.

I am not prepared at the present time to go into a full discussion of the probable affinities of the Isoetaceae, but I do wish to emphasize some of the striking similarities existing between the embryogeny of *Isoetes* and that

of *Selaginella*. To illustrate some of these resemblances, we may take the embryo of *Selaginella spinulosa* as illustrated by Bower (3, fig. 190) and imagine the modest suspensor removed. Then suppose that the first cotyledon, the lower one, should develop rapidly while the second, the upper one, is greatly delayed. (This is actually the case to some extent!) And now, if the lower side were enlarged into a more prominent foot, the result would be an excellent likeness of an *Isoetes* embryo. Orientation of embryos in the two forms is exactly the same without any change whatever, and the presence of rhizoids on both gametophytes makes the resemblance still more striking. Campbell's figure (6, fig. 299) illustrating steps in the development of *Selaginella martensii* may also be quite as strikingly compared. Although the gametophyte is not shown in connection with the embryo, it is interesting to note that the foot actually develops in *S. martensii* and that the lower end of the embryo first grows toward the center of the prothallium and then turns to one side so that the embryo becomes parallel to the apical surface of the prothallium, just exactly as it does in the case of *I. lithophila*.

In Bower's attempt (3) to compare the embryogeny of *Isoetes* (based, undoubtedly, upon the interpretations of Campbell) with that of *Selaginella* and the Lycopodiaceae he offers the following suggestion: "The suspensor (in *Isoetes*) is entirely absent, and the embryo, composed only of the two tiers corresponding to those of the other Lycopods, is usually oriented so that its apex is from the first directed towards the neck of the archegonium. That the rotation necessary to bring this about may occur is indicated by the differences of position of the basal wall noted by Campbell."

If my thesis regarding the early orientation of the embryo is correct, it becomes unnecessary to assume a rotation before the embryo takes on some definite form by which it may be followed in its orientation and development. Once this is thoroughly substantiated, therefore, it should lend strength to Bower's arguments and make at least one of his difficult assumptions unnecessary in justification of including *Isoetes* in the order Lycopodiales more or less closely associated with *Selaginella*. It must be admitted, however, that there are still serious objections to be overcome before this classification can be definitely accepted, especially in the light of comparatively recent seriological investigations. Conradi (7), for example, has shown that seriological reactions do not suggest close taxonomic relationships between the families these genera represent, but it is not certain that this means of determining such relationships is unfailingly accurate.

SUMMARY

1. The ripe megaspore of *Isoetes lithophila* contains a single nucleus which is located in the base of the spore.

2. The mature female gametophyte is composed of rather compact, small-celled tissue in the apical region and comparatively loose, food-storage tissue in the basal portion. The change from one type of tissue to the other is

often quite abrupt, but nothing comparable to a diaphragm is in evidence.

3. The number of archegonia produced by the prothallium, when fertilization is prevented, seems to be indefinite in that new ones are formed continuously until the food supply of the gametophyte is exhausted and death ensues.

4. The prominence of rhizoids on the mature prothallium is a striking feature. These rhizoids have their origin in the superficial cells of the apical region on the surface exposed by splitting of the spore coat.

5. A conspicuous calyptra encloses the first leaf of the embryo as it protrudes from the gametophyte; after the leaf breaks through, the calyptra remains as a sheath about the base of the leaf until the gametophyte disintegrates.

6. The embryo first elongates toward the center of the prothallium, away from the neck of its archegonium. The lower, pointed end nearest the center of the prothallium can be recognized very early as the primordium of the first leaf.

7. The elongated embryo shifts as it grows until its long axis comes to lie roughly parallel to the apical surface of the gametophyte.

8. This shift in orientation of the embryo makes it appear that the embryogeny of *Isoetes* is similar in all essentials to that of *Selaginella*.

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MANGANESE AND THE GROWTH OF LEMNACEAE

ALBERT SAEGER

During the course of studies on the nutrition of *Spirodela polyrrhiza* and species of *Lemna,* conducted through a period of years, it was observed occasionally that the plants deteriorated and at last failed to grow when kept in Knop's solution of dilute concentration. This deterioration and failure to grow usually occurred toward the end of experiments covering a period of weeks or months. Sooner or later, usually after another stock solution with different lots of chemicals had been prepared, the plants would recover rapidly and resume vigorous growth. The salts used were sometimes the " chemically pure " grade from supply houses and sometimes recrystallized salts.

It was necessary to determine the cause of this occasional failure of growth. A number of investigators have claimed that certain elements not usually added to nutrient solutions are essential in small amounts to the plants. It has been reported that the absence of manganese, boron, zinc, copper, iodine, silicon, aluminum, fluorine, and other elements may be a deciding factor in the growth of plants in culture solutions. Recently Hopkins (1930) has demonstrated the necessity of manganese for *Chlorella* sp. and later (1931) for *Lemna minor.* Clark and Fly (1930), however, concluded that there was no indication that manganese is an essential element in the nutrition of *Spirodela polyrrhiza.*

To determine whether manganese is necessary in a synthetic nutrient solution for Lemnaceae, experiments were made with five species. A preliminary test indicated that at least some of the species would react unfavorably when this element was lacking from a nutrient solution.

EXPERIMENTS AND RESULTS

Experiment 1. Effect of the absence of manganese on the growth of Lemnaceae

Two cultures of *Spirodela polyrrhiza* (L.) Schleiden were prepared, 25 plants per culture, in a dilute Knop's solution containing 1.0 mg. iron (as FeCl$_3$) per liter, and two similar cultures containing in addition 1.0 mg. manganese (as MnSO$_4$) per liter. In the same manner cultures of *Spirodela oligorrhiza* Kurz, *Lemna minor* L., *L. valdiviana* Philippi, and *L. minima* Philippi were provided, in duplicate, both with and without manganese. The plants used had grown for 2 weeks in a mineral nutrient solution. The

experiment covered the period Nov. 3, 1931, to Jan. 5, 1932. The following stock solutions were employed:

1. Knop's solution (modified) :

$Ca(NO_3)_2.4H_2O$	5.00 grams
KNO_3	1.67 grams
KH_2PO_4	1.67 grams
$MgSO_4.7H_2O$	1.67 grams
Redistilled water	1000 cc.

For use this solution was diluted 100 times. The chemicals were recrystallized from redistilled water from three to five times, according to original purity.

2. Iron solution: 4.84 g. $FeCl_3.6H_2O$ (Kahlbaum's) were dissolved in a liter of redistilled water. This solution is stable for several weeks if kept in the dark at 5° C. One cc. contains one milligram iron. A test for manganese on several grams of the salt showed no detectable amount present.

3. Manganese solution: 4.06 g. $MnSO_4.4H_2O$ (Merck's "highest purity") were dissolved in one liter of redistilled water. One cc. contains one milligram of manganese.

No precipitate appeared in any of these stock solutions. All water used for the solutions was thrice distilled, the second distillation from an acid $KMnO_4$ solution, the third from $Ba(OH)_2$ solution, using a Pyrex still. All glassware which came in contact with the culture solutions was Pyrex and was cleaned with chromic acid cleaning solution, rinsed six times with clear water, three times with distilled, and twice with redistilled water. During each transfer to a new culture solution the same cleaning procedure was followed. Erlenmeyer flasks of 500-cc. capacity, each with 250 cc. solution, were used for the cultures. To avoid crowding of the plants, one-liter flasks were occasionally used. The flasks were protected from dust by hardened filter-paper covers held in place with rubber bands.

The cultures were placed in a water bath with a Cenco centrifugal stirrer, cooling coils, and automatically controlled immersed heaters. The temperature was maintained, with minor interruptions, at 25° C. Illumination was provided by two 500-watt Mazda bulbs with aluminum reflectors, placed at an angle of 45 degrees near the edge of the bath, for 9½ hours daily. The positions of the cultures were changed daily. The plants were transferred to new solutions twice a week. The number of plants, weekly percentage increase in number of plants, total dry weight, dry weight per 100 plants, and the H-ion concentration of the solutions were determined as indicated in tables 1 to 5. When the number of plants increased beyond the capacity of the vessels, the number was reduced. usually to 25. Thereafter the total number was calculated from the weekly percentage increase.

After 24 days specimens of *S. oligorrhiza* from cultures with manganese and from cultures without manganese were photographed (pl. 9). Similarly plants of *S. polyrrhiza* were photographed after 49 days (pl. 10). The symptoms of manganese deficiency were first observed in *S. oligorrhiza* and in *L. minor* after 2 weeks. The response to the lack of manganese was distinct and characteristic. The youngest leaves remained very small and

24

later died. Sometimes the tips died first, but often there appeared bands of necrotic areas lying perpendicular to the midvein. Sometimes several parallel bands appeared on one leaf. The larger, older leaves which had been produced before the deficiency became acute were the last to die, so that at one period the plants consisted of large, apparently healthy leaves to which very small dead or partly dead younger leaves were attached (pl. 10). The earliest indication of manganese deficiency was seen in a great decrease in

TABLE 1. *Effect of absence of manganese. Number of plants*

Age in days	Spirodela polyrrhiza		Spirodela oligorrhiza		Lemna minor		Lemna valdiviana		Lemna minima	
	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn
0	50	50	50	50	50	50	50	50	50	50
7	88	92	85	96	85	74	88	88	125	116
14	140	126	150	103	156	138	188	173	225	205
21	323	225	452	151	548	364	582	502	705	650
28*	963	648	1880	305	1677	866	3504	1516	3751	2496
35	3043	1983	5565	1712	4899	1485	8610	3655	13560	7878
42	6390	3650			Discontinued				53160	25430
49	17250	7330							211450	101200
56	34070	11550							713000	320300
63	90630	26100							3008900	909800
Ratio final no.	3.5	1	3.2	1	3.3	1	2.3	1	3.3	1
Generation time (days)†	5.8	7.0	5.3	6.9	5.3	7.2	4.7	5.7	4.0	4.5

* Total number after 28th day calculated from the weekly percentage increase.

† Calculated for the entire period of the experiment from the relation (Buchanan and Fulmer, 1928):

$$\frac{t \log 2}{\log b - \log B},$$

where t = time (days), b = no. plants after time t, and B = no. plants at beginning.

TABLE 2. *Effect of absence of manganese. Percentage increase in number of plants*

Age in days	Spirodela polyrrhiza		Spirodela oligorrhiza		Lemna minor		Lemna valdiviana		Lemna minima	
	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn
7	76	84	70	92	70	48	76	76	150	132
14	59	37	77	7	84	87	114	97	80	77
21	131	79	201	47	251	164	209	190	213	217
28	198	188	316	102	206	138	502	202	433	284
35	216	216	196	462*	199	71	143	141	262	216
42	110	84			Discontinued				292	225
49	170	101							296	299
56	142	108							237	216
63	166	126							322	184

▸ High count due to breaking apart of injured plants.

TABLE 3. *Effect of absence of manganese. Dry weight of all plants (milligrams). After 21st day, calculated from sample weighings and data of table 1*

Age in days	Spirodela polyrrhiza		Spirodela oligorrhiza		Lemna minor		Lemna valdiviana		Lemna minima	
	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn
21	133	127	79	35	73	38	54	33	61	51
35	1358	970	1549	154	1153	126	862	197	—	—
56	13501	6919			Discontinued				—	—
63	74610	7839							372530	44020
Ratio (final)	9.5	1	10.0	1	9.1	1	4.4	1	8.5	1

TABLE 4. *Dry weight per 100 plants (milligrams)*

Age in days	Spirodela polyrrhiza		Spirodela oligorrhiza		Lemna minor		Lemna valdiviana		Lemna minima	
	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn	+ Mn	− Mn
21	41.3	56.4	17.4	23.2	13.3	10.5	9.2	6.5	8.6	7.7
35	44.6	48.9	27.8	9.0	23.5	8.5	10.1	5.4	—	—
56	39.6	59.8			Discontinued				—	—
63	82.3	30.0							12.4	4.8
Ratio (final)	2.7	1	3.1	1	2.8	1	1.9	1	2.6	1

TABLE 5. *Hydrogen-ion concentration of culture solutions (quinhydrone electrode)*

pH

Dilute Knop's solution, plus one mg. Fe per liter (FeCl₃) ....

Dilute Knop's solution, plus one mg. Fe per liter, plus one mg. Mn per liter (MnSO₄) 4.4

Dilute Knop's solution, in which plants had been growing for 3½ days (250 cc. solution)

Species	Mn	Number of plants in culture vessel	pH
S. polyrrhiza	+ Mn	40	5.6
		39	5.6
	− Mn	45	5.8
		47	5.8
S. oligorrhiza	+ Mn	43	5.0
		42	4.8
	− Mn	51	4.8
		45	4.8
L. minor	+ Mn	43	4.7
		42	4.8
	− Mn	43	4.8
		31	4.8
L. valdiviana	+ Mn	46	4.6
		42	4.7
	− Mn	44	4.6
		44	4.6
L. minima	+ Mn	65	4.6
		60	4.6
	− Mn	59	4.6
		57	4.6

size of the youngest leaves, and a decrease in root length, as compared with the controls.

Definite symptoms appeared in *L. valdiviana* after 3 weeks and in *L. minima* after 8 weeks. *S. polyrrhiza*, both with and without manganese, grew well and remained healthy for 7 weeks. At times the cultures without manganese seemed better than those with manganese, probably indicating that 1.0 mg. manganese per liter exceeds the optimum concentration (see exp. 3). The relatively sudden appearance of manganese-deficiency symptoms was very striking. Within 3 days one could observe in *S. polyrrhiza* the change from an apparently healthy, vigorously growing condition to one showing all the acute symptoms described: short roots, small leaves, necrotic bands, and almost complete cessation of growth.

The data in tables 3 and 4 show quantitatively the results just described. *S. oligorrhiza* and *L. minor* showed a significant difference in dry weight within 3 weeks, *L. valdiviana* within 5 weeks, *S. polyrrhiza* within 8 weeks, and *L. minima* within 9 weeks. The cultures containing manganese produced from 4.4 to 10 times as much dry matter as those without manganese. At the time the different cultures were discontinued, growth in the solutions without manganese had practically ceased, while it was continuing as before in those with manganese.

The data in table 4 give an idea of the size of the individual plants. The term " plant " is here used to refer to a group of from 2 to usually not more than 8 leaves attached to each other by short connecting stalks. In obtaining dry weights of the manganese-deficient plants, it was necessary to include those leaves that had grown before the symptoms had appeared. The dry weight of only those formed after the symptoms had appeared would have been very minute, but it was difficult to separate such leaves from the older ones. Reference to plate 10 will make this point clear.

The data and observations show clearly that when manganese is withheld from cultures of *Spirodela* and *Lemna* kept in Knop's solution, the cultures fail to continue their growth and finally die. Hopkins' (1931) work with *Lemna minor*, then, has been confirmed and extended, and the conclusions of Clark and Fly (1931) with respect to the essential nature of manganese cannot be accepted.

Experiment 2. Time for recovery after addition of manganese and time for deterioration after removal of manganese

As indicated in experiment 1, *S. oligorrhiza* showed distinct injury after it had been in a solution lacking manganese for 3 weeks. At this time duplicate samples of 25 plants were transferred from the minus manganese cultures to plus manganese solutions (1.0 mg. per liter), and similarly plants from plus manganese cultures were transferred to minus manganese solutions. They are called, respectively, " — + " and " + — " cultures. The methods followed were like those of experiment 1.

After 3 days the "— +" cultures showed distinct signs of recovery. The leaves which had ceased growth owing to lack of manganese now produced new, green, and rapidly growing daughter leaves. These continued their vigorous growth, producing other leaves. After 2 weeks all the old manganese-deficient leaves had dropped off and the new plants were not distinguishable from those in the plus manganese cultures. The " + —" cultures, however, did not show noticeable injury owing to lack of manganese until after 5 weeks. Then they deteriorated very rapidly, with the typical deficiency symptoms described above.

Similarly, manganese was added to the cultures of the other four species of Lemnaceae mentioned in experiment 1, after they had developed the symptoms of manganese deficiency. In each instance the recovery was evident 3 days after manganese had been added. The data for *S. oligorrhiza* are given in table 6.

These experiments further indicate the necessity of manganese for the growth of the plants.

TABLE 6. *Recovery and deterioration of S. oligorrhiza with addition or removal of manganese*

Age in days	Number of plants *		Total dry weight (mg.)		Dry weight per 100 (mg.)	
	— + †	+ — †	— +	+ —	— +	+ —
0	50	50	4.5	13.9	9.0	27.8
7	269	200				
14	1103	1104	164	195	14.9	15.0
21	2846	1811				
28	5625	5676				
35	61986	20641				
42	140050	70545	53500	17150	38.2	24.3
Generation time (days)	3.7	4.0				

* Calculated from weekly percentage increase.
† — + indicates cultures grown without manganese, then transferred to solutions with manganese. + — indicates cultures grown with manganese, then transferred to solutions without manganese.

Experiment 3. Concentration of manganese in nutrient solutions

Observation of the cultures in experiment 1 indicated that a concentration of 1.0 mg. manganese per liter was probably above the optimum for growth in the dilute Knop's solution. A series of duplicate cultures containing 10 plants each of *S. polyrrhiza* was now prepared, to which were added, respectively, 0.001, 0.01, 0.1, and 0.5 mg. manganese (as $MnSO_4$) per liter. The methods of experiment 1 were followed, except that the daily period of illumination was increased to 15½ hours. The plants used had been growing without manganese for 7 weeks and had the appearance of the

manganese-deficient plants shown in plate 10. Cultures of these were also kept as controls.

After 3 days the cultures containing 0.001, 0.01, 0.1, and 0.5 mg. manganese per liter were resuming growth. Vigorous young leaves were growing out of the older leaves and out of the injured smaller leaves. The controls lacking manganese showed no recovery at all. There was not much difference in the appearance of the plants which received 0.5 mg. and those which received 0.001 mg. per liter. The experiment was concluded after 13 days, at which time nearly all the plants in the solution without manganese were dead. Data are given in table 7.

TABLE 7. *Concentration of manganese necessary for recovery of S. polyrrhiza from manganese deficiency. Age of cultures, 13 days*

Milligrams manganese added per liter	Number of plants		Generation time (days)	Total dry weight (mg.)	Dry weight per 100 plants (mg.)
	Initial	After 13 days			
None	20	40	14.0	9.5	2.4
0.001	20	104	5.9	54.6	52.5
0.01	20	122	5.4	54.5	44.8
0.1	20	124	5.3	57.7	46.5
0.5	20	98	6.1	57.2	58.4

The data indicate that 0.001 mg. per liter of manganese (one part per billion) is sufficient to bring about rapid recovery and to maintain vigorous growth of *S. polyrrhiza* in a dilute Knop's solution, under the conditions of this experiment.

Another series, in which 0.001 and 0.000001 mg. manganese per liter were added, showed again that the former concentration was sufficient for recovery and maintenance. But the cultures with 0.000001 mg. per liter showed no signs of recovery, even after 17 days.

In maintaining stock cultures of the other four species, it was found that 0.001 mg. of manganese per liter is sufficient to maintain good growth and better than a concentration of 1.0 mg. per liter. But plants in such cultures, apparently do not contain much reserve manganese, as the results of experiment 7 indicate.

Experiment 4. Substitution of other elements for manganese

S. polyrrhiza, S. oligorrhiza, and *L. minor,* which had been grown in a solution lacking manganese until definite deficiency symptoms had developed, were placed in Knop's solution containing 0.001 mg. per liter of each of the following elements: aluminum, boron, copper, fluorine, iodine, and zinc. None of the plants recovered. These elements, therefore, cannot take the place of manganese, when each is supplied in the concentration which furnishes a satisfactory amount of manganese.

Experiment 5.   Manganese as an impurity in iron

In a previous paper a method was described of obtaining pure cultures of *S. polyrrhiza* (Saeger, 1930). There it was stated (p. 119) that the plants growing in a dilute Knop's solution which had been sterilized became chlorotic, and that this chlorosis was prevented by the addition of a piece of iron wire to the solution. The iron, of course, rusted, but the sterile plants thrived in such a solution for weeks without being transferred. It is now believed that the iron wire contributed one or more elements besides iron to the solution. The following experiment shows that the "iron" probably contributed manganese to the solution.

S. polyrrhiza was grown in Knop's solution lacking manganese until there was no further growth and most of the young leaves were dead. Ten such plants were added to each of four flasks containing 250 cc. Knop's solution minus manganese. A small piece of iron (a tack, about 0.2 g. of metal) was added to two of the cultures. After 5 days the plants in the cultures with the piece of iron were beginning to produce new leaves, and after 9 days the newly formed leaves were large and green, while the controls showed no recovery. The plants, however, did not recover as rapidly as they did when 0.001 mg. manganese was added per liter. The periodate test showed that manganese was present in the solutions containing the piece of iron. Manganese was also found in a sample of iron wire commonly used for analytical purposes. It remains to be demonstrated whether the "iron" contributed other elements to the solution—e.g., copper or silicon.

Experiment 6.   Production of plants deficient in manganese for
experimental use

S. polyrrhiza, L. minor, L. valdiviana, and *L. minima,* retained from the cultures of experiment 1, all of which were showing symptoms of manganese deficiency, were allowed to recover in Knop's solution plus only 0.001 mg. manganese per liter, under a daily period of illumination of 15½ hours. After 2 weeks, when the plants were in a state of vigorous growth, they were again transferred to a solution without manganese.

The first two species showed symptoms of manganese deficiency 5 days later and the last two 9 days later. The results indicate that little manganese was absorbed during the 2 weeks when a minute amount of the element was supplied.

With this technique an easy method is available for obtaining within a few days plants deficient in manganese. Such plants were used in experiments to be reported later.

Experiment 7.   Effect of different concentrations of manganese in the nu-
trient solution upon the time required for development of deficiency
symptoms after manganese is withdrawn

In one instance *S. polyrrhiza* developed distinct deficiency symptoms about 7 weeks after manganese had been withheld from the cultures (exp. 1).

whereas in another instance these symptoms appeared within 5 days after the withdrawal of manganese (exp. 6). It seemed probable that in the former instance the residual supply of manganese in the plants must have been greater than in the latter.

The following cultures were begun to determine if this species can accumulate from the nutrient solution a supply of manganese in excess of that essential for growth, and whether such residual manganese becomes available to the growing tissue after manganese has been withdrawn from the substrate. The modified Knop's solution with 1.0 mg. iron per liter was used, but with different amounts of manganese: *A*. Without manganese. *B*. With 0.001 mg. manganese per liter. *C*. With 1.0 mg. manganese per liter.

All cultures were in duplicate. Into each flask were placed ten plants which had been maintained in the absence of manganese until growth had ceased entirely. The other conditions were the same as those in experiment 3. Observations were made as indicated in table 8.

TABLE 8. *Recovery of S. polyrrhiza from manganese deficiency*

Age in days	*A* Minus Mn	*B* 0.001 mg. Mn per liter	*C* 1.0 mg. Mn per liter
0	20 plants No growth	20 plants No growth	20 plants No growth
3	No growth	Few new leaves forming	Few new leaves forming
7	No growth	Many new leaves	Many new leaves
11	Nearly all dead	Appear fully recovered	Appear fully recovered
14	20 plants All dead	64 plants, green and healthy looking	73 plants, green and healthy looking, but leaves not as large as those in *B*
17	—	127 plants 63.5 mg. dry weight *	104 plants 62.0 mg. dry weight *
22	—	257 plants 155.6 mg. dry weight * 60.5 mg. per 100	210 plants 88.2 mg. dry weight * 42.0 mg. per 100

* Estimated from weight of one-half number of plants.

After cultures *B* and *C* had been growing in the presence of manganese for 22 days, thirty plants from each series were selected and transferred to a similar solution containing no manganese, ten plants per flask. They were watched daily for the appearance of manganese-deficiency symptoms. Twice a week they were transferred to fresh solutions. After 3 days typical symptoms appeared in the cultures which had previously been grown with 0.001 mg. manganese per liter. The young leaves remained small, turned brown, and died. None of the cultures which had been grown with 1.0 mg. manganese per liter showed any deficiency symptoms until 24 days had elapsed. After this they deteriorated rapidly, and in 28 days growth had ceased and the young leaves were dead.

The data show, then, that *S. polyrrhiza* can accumulate a reserve supply of manganese when this element is available in sufficient concentration in the substrate. This supply is no doubt transferred to the newly developing leaves when manganese is lacking in the substrate. It has not been determined to what extent the plants accumulate manganese from natural waters. The manganese content of natural waters is being investigated.

<div align="center">DISCUSSION</div>

The necessity of manganese for the growth of Lemnaceae has been demonstrated in these experiments. The occasional failure of growth observed during the course of earlier experiments, when known amounts of manganese were not added to the nutrient solutions, was no doubt due to the absence of this element, and perhaps of others, from the culture solutions.

Even when plants are grown in solutions made with recrystallized chemicals, containing no manganese, failure of growth does not necessarily occur unless the experiment is continued long enough to exhaust the residual supply of manganese introduced in the original plants. The results of experiment 7 indicate that plants grown in the presence of manganese can accumulate a supply of this element sufficient to allow reproduction of a considerable number of daughter plants, before any deficiency becomes evident. It is important, then, to know the previous history of plants when they are to be used in experiments to determine the effect on growth of minute traces of elements.

Since it has been demonstrated that only 0.001 mg. manganese per liter in the solution is sufficient to maintain vigorous growth, great precautions must be taken to guard against the introduction of even minute traces of the element under investigation, before concluding that this element is not essential.

The addition of manganese had practically no effect upon the H-ion concentration of the solutions, indicating that such a factor could not account for the recovery obtained by the addition of manganese. It was found that the solutions in every case became more alkaline while the plants were growing in them, but there was no significant difference in reaction between those with and those without manganese (table 5). *S. polyrrhiza*, the largest of the species, produced the greatest change toward alkalinity.

It is possible that some other element may be substituted for manganese. But aluminum, boron, copper, fluorine, iodine, and zinc were each tested in a concentration of 0.001 mg. per liter, and none of these elements would bring about recovery of manganese-deficient plants.

With the data at hand, one may consider the rôle of manganese in natural waters. Its absence from any body of water—or its presence in too low a concentration—would prevent the growth of Lemnaceae. It is quite likely that manganese may be a limiting factor in the growth, not only of these plants, but also of algae, diatoms, bacteria, and perhaps protozoa. Hopkins

(1930) has demonstrated that it is essential for *Chlorella,* and no doubt it is essential for others. Since Uspenski (1927) has shown that the iron content of natural waters varies greatly with seasonal changes, it may be assumed that the same is true for manganese. Uspenski has also shown that the concentration of available iron in waters may be critical for the occurrence or absence of particular species of algae. Since it has been shown that manganese is essential in small amounts for aquatic plants, the problem of their distribution with respect to this element becomes one of great interest. In this connection information must be obtained about the effects of other conditions upon the solubility and availability of manganese: effects of light, of H-ion concentration, of organic substances, and of elements which might be antagonistic to manganese.

It has been shown that exceedingly minute quantities of manganese can bring about a remarkable stimulation (or resumption) of growth in cultures which previously lacked this element. Stimulation of growth of Lemnaceae by the addition of small amounts of organic matter has been reported by a number of investigators. One may ask whether increase in growth may not have resulted from the addition of small amounts of essential elements— e.g., manganese.

Experiments to be reported in detail later have shown that extracts of autolyzed yeast, raw carrot, spinach, *Lemna* leaves, and leaves of *Digitalis purpurea* added to a nutrient solution will stimulate the growth of manganese-deficient plants. In fact, a biological method for determining the presence of exceedingly minute amounts of manganese in various extracts has been devised, and this method may be roughly quantitative when the element is present in high dilution. These experiments have also indicated that manganese may be one of the constituents of the group of substances which Bottomley termed "auximones," although it is not likely that the beneficial effect of adding extracts of organic matter to nutrient solutions can be entirely explained by the presence of traces of essential inorganic elements.

SUMMARY

1. Manganese is essential for the growth of five species of Lemnaceae. (*a*) When these plants are deprived of manganese, growth ceases after a time, and typical symptoms of the deficiency are observed. (*b*) When plants showing symptoms of manganese deficiency are again supplied with the element, recovery may be observed readily within 3 days.

2. A supply of 0.001 mg. manganese per liter of solution (one part per billion) was sufficient to maintain vigorous growth under the conditions specified.

3. One mg. manganese per liter seems to be too high a concentration for optimum growth.

4. Aluminum, boron, copper, fluorine, iodine, or zinc, when each is supplied in a concentration of 0.001 mg. per liter, cannot replace manganese.
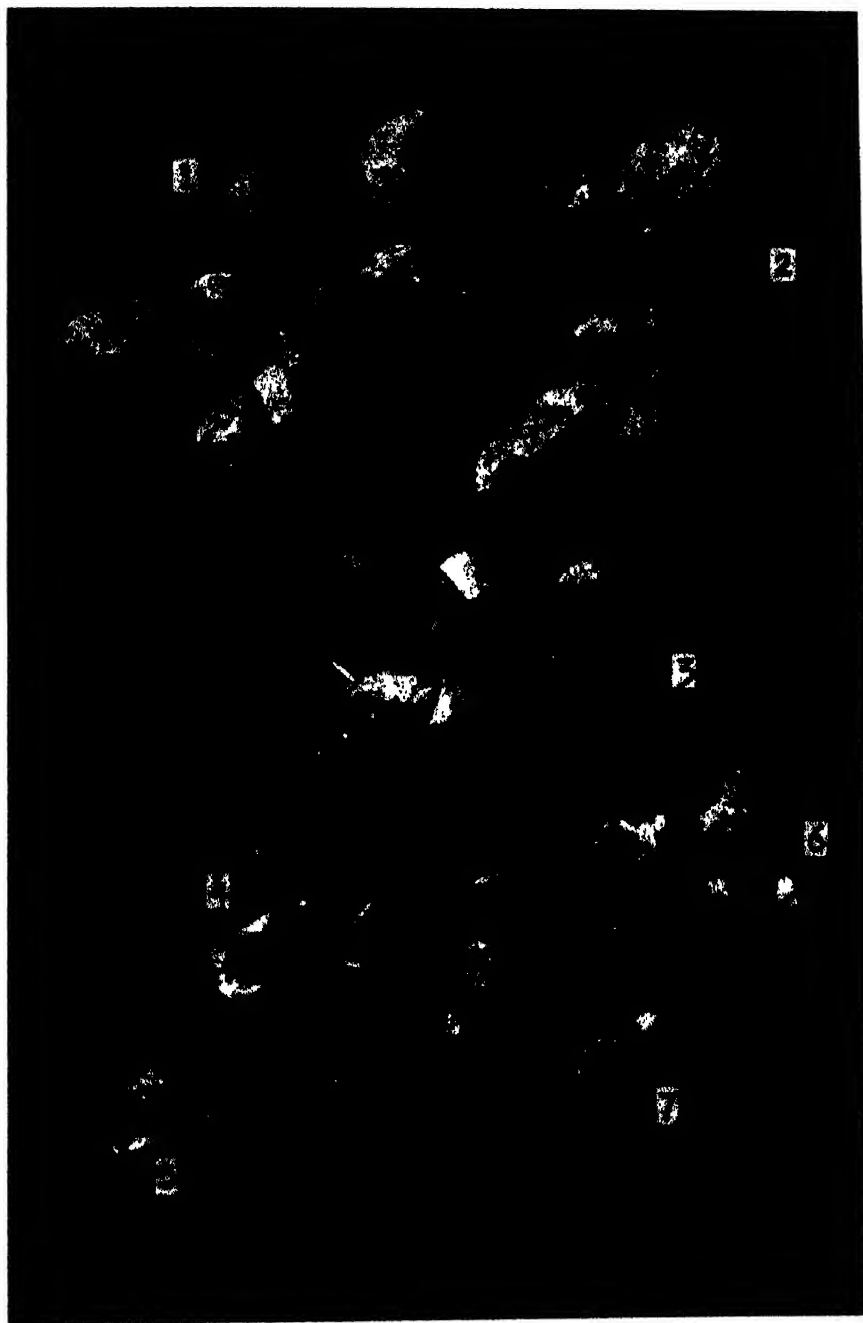
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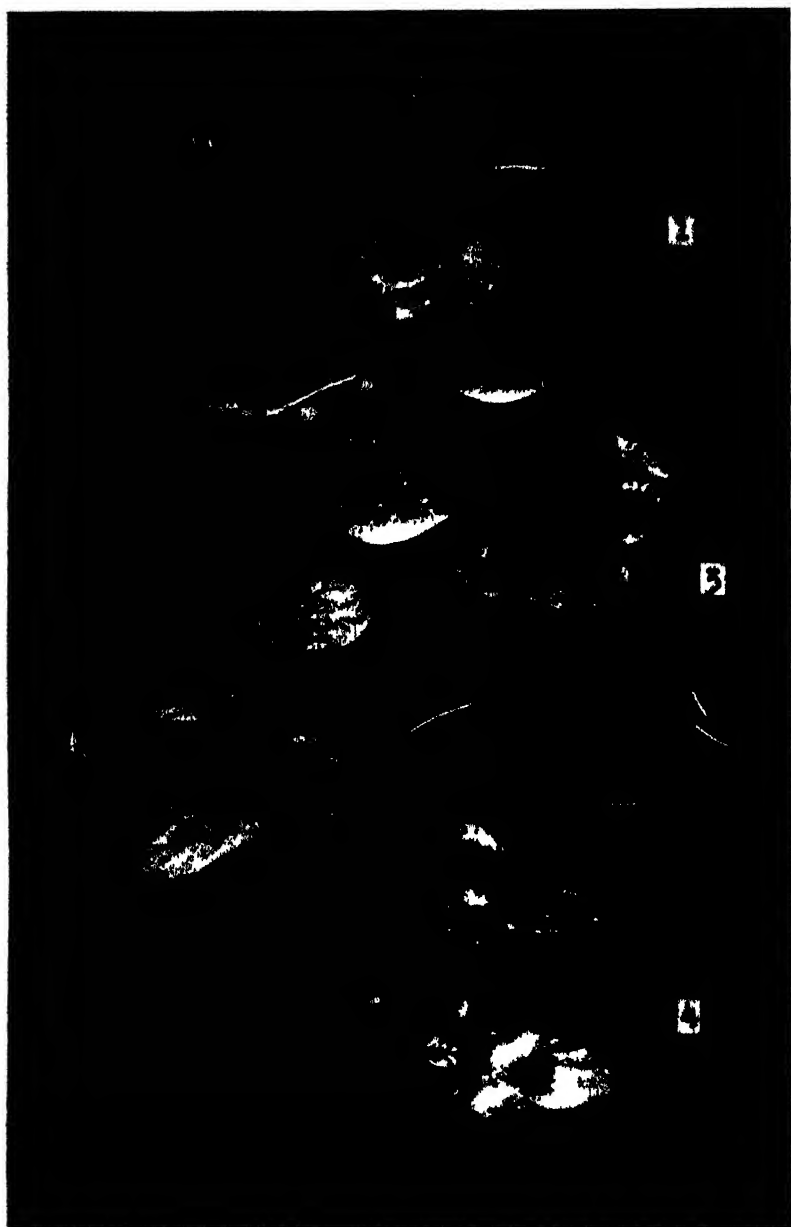
SAEGER: LEMNACEAE

5. When chemicals of questionable purity are used in nutrient solutions, sufficient manganese is probably added as an impurity in the chemicals.

6. A method has been given for transforming vigorously growing plants of *S. polyrrhiza* into plants showing definite symptoms of manganese deficiency within an experimental period of from 3 to 5 days.

7. Lemnaceae can accumulate a reserve supply of manganese when the element is supplied in sufficient concentration.

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EXPLANATION OF PLATES

PLATE 9

Spirodela oligorrhiza. Fig. 1, 2, 3, plus manganese. Fig. 4, 5, 6, 7, minus manganese. Age of cultures, 24 days.

PLATE 10

Spirodela polyrrhiza. Fig. 1, plus manganese. Fig. 2, 3, 4, minus manganese. Age of cultures, 49 days.

THE DISTRIBUTION OF FUNGI AS COMPARED WITH THAT OF PHANEROGAMS

G. R. BISBY

Phanerogamic botanists have determined the approximate distribution over the earth of many thousands of species of vascular plants. Students of plant geography have been able to utilize this knowledge to formulate principles of distribution and spread of higher plants, to determine the effects of barriers and climates, and even to develop hypotheses as to the age, origin, and evolution of plants.

Mycologists have been able to map with accuracy the geographical distribution of comparatively few fungi: these are principally species parasitic upon cultivated plants, and their spread has been assisted by man. Obligate parasites, such as the Uredinales, can of course have a distribution no greater than that of their hosts; and even many obligate saprophytes are limited to the dead parts of certain vascular plants, so that their distribution also depends in part upon the distribution of the higher plants supplying the substratum. It is known in a general way that certain fungi are confined to tropical regions, others to temperate or even to arctic regions; that some fungi have a wide distribution, others a restricted one that apparently may be limited even to the type locality. Several authors from Schroeter (17) to Gilbert (7) have dealt with other ecological relationships of the fungi. The inadequacy of the knowledge of the exact distribution over the earth of most species of fungi, however, has largely precluded the formulation of principles and hypotheses regarding the distribution and spread of the fungi. The writer has assisted in efforts to determine the distribution of the Uredinales (1) and the fungi of Manitoba (2) and of India (5). An attempt will be made here to carry further an analysis of the fungi, exclusive of lichens and bacteria.

Fungi have been present upon the earth for a very long time,[1] phanerogams for a much briefer period. As plants and animals gradually evolved, fungi came to be associated predominantly with seed plants and their remains. This is evident from table 1, which represents a summary of Seymour's Host Index (20).

[1] Seward (19, p. 427) states: "One thing is clear: from the Devonian period onwards and even from a more remote age there were parasitic and saprophytic fungi. . . . We can safely say that bacteria and many other fungi are entitled to be included among the most ancient members of the plant kingdom."

Table 1 was prepared from a count of the entries, excluding synonyms, except that the number of fungi upon Dicotyledoneae was computed from average pages. It may be observed that about 94 per cent of the fungi included are recorded upon seed plants. The Gymnospermae, which are all trees or shrubs, have the largest average number of fungi per host. The woody Dicotyledoneae support larger average numbers of fungi than the herbaceous. Although Seymour records 11,000 hosts, there are many species and even genera of plants in North America which have not yet been reported

TABLE I. *An analysis of the fungi and hosts recorded from North America*

Group of hosts	Approximate numbers of				
	Host entries	Fungus entries	Total pages	Average hosts per page	Average fungi per host
Algae	53	72	1 1/2	35	1 1/3
Fungi	473	808	14	34	1 3/4
Lichens	57	88	1 3/4	33	1 1/2
Bryophyta	44	118	2	22	2 3/4
Pteridophyta	111	249	4 1/2	25	2 1/4
Gymnospermae	184	3733	52	3 1/2	20 1/4
Monocotyledoneae	1861	6454	107	17 1/2	3 1/2
Dicotyledoneae	7527	c. 30000	571	14 3/4	c. 4
Animalia	772	1110	21	37	1 1/2
Totals	11082	c. 42600			

as hosts for fungi. The well-known fact that the animal kingdom is relatively free from the attack of fungi is indicated by the references in Seymour's book, which has only 21 pages devoted to Animalia, nearly 20 of which refer to insects. The ancient Algae, Bryophyta, and Pteridophyta appear to have reached the present era with comparatively few fungi definitely associated with them as parasites or saprophytes, although the North American records are still very incomplete.

About four times as many fungi as hosts are listed by Seymour, but of course very many of the fungi are entered two or more times; the total number of definite species of fungi in the book is probably not very different from the number of hosts. It must be remembered, however, that many saprophytic fungi cannot be included in a host index. There are probably more fungi than seed plants recorded in North America.[2]

Since fungi are predominantly associated with seed plants, as saprophytes and parasites, a comparison of the numbers and distribution of the two groups will be attempted. The total number of species of Spermatophyta cannot be stated with accuracy, but apparently about 160,000 species are known, and perhaps 200,000 may exist. The number of species of fungi present over the whole earth can scarcely be estimated as yet. About 100,000 specific names have been applied to the fungi, but many of these names mean little.

[2] Oudemans' *Enumeratio systematica fungorum* would probably give similar but somewhat more complete data, for the fungi recorded in Europe.

About half the specific names of the fungi have been proposed during the past fifty years, and many more must still be applied to new species; but on the other hand, many old names will prove to be synonyms or need to be dropped. The number of species of fungi on earth is evidently of the same order as that of the number of species of seed plants: it is possible that 200,000 species of fungi may eventually be found. At the present time, however, the known seed plants outnumber the known fungi by about two to one.

Despite the smaller total number of species of fungi known, more fungi than phanerogams have been reported from various areas. It is suggested above that there are probably more fungi than seed plants recorded in North America. It is certainly true that smaller areas, such as states or provinces, contain more fungi than phanerogams: there are, for example, more than twice as many fungi as spermatophytes in Manitoba (2). A similar ratio exists in the countries of Europe (see 2, 5). Lind (10) notes that only about 1400 phanerogams occur in Denmark, but he records 3324 fungi. This is a ratio of 19 fungi to 8 phanerogams. In areas extending into the tropics, such as India (5), there are more seed plants than fungi recorded, but fungi are very inadequately known in tropical or subtropical parts of the world.

As we have previously reported (2), nearly 60 per cent of the species of fungi known in Manitoba appear to occur in Europe, whereas less than 30 per cent of the phanerogams growing without cultivation in Manitoba occur in the wild state in Europe, and only about 20 per cent of the native species of spermatophytes of Manitoba are native also to Europe. A further analysis of the fungus flora of Manitoba as far as known to January, 1932, yields the following data: about 2100 species of fungi (including Myxomycetes) are known in the province of Manitoba, and 1122 phanerogams growing without cultivation in the same area. Only 170 of the fungi have been found exclusively upon cultivated plants. About 55 per cent of the fungi apparently native to Manitoba are known, under the same names, as natives of Europe. An area of about one square mile along the Red River just south of Winnipeg, which has been carefully surveyed, has yielded 1280 species of fungi, only 141 of which were found associated exclusively with cultivated plants. In this square mile 354 species of phanerogams have been found growing without cultivation.

The data just presented indicate that species of fungi in general have a wider distribution than species of spermatophytes. A comparison of the floras of India and Europe serves to strengthen this conclusion (5). Only about 6 per cent of the indigenous phanerogams of India are also indigenous to Europe, but 23 per cent of the species of fungi recorded from India appear to be the same as species occurring in Europe.

The figures and data in this paper are presented with a full realization of the uncertainties involved. Many fungi have been given old names when they were really new species; but such cases are neutralized in a statistical comparison by the cases of application of new names to fungi that were not

new. It might also be argued that in comparing America or Asia with Europe, we give the seed plants different specific names, although they have often much similarity, as indicated by the common generic names; but we are more apt to consider the fungi identical species. In other words, were the fungi as large as seed plants, we might see specific differences. The following, however, must be remembered: that we do magnify the fungi with the microscope; that the majority of fungi are not highly specialized as to food requirement, so that, for example, a saprophyte or parasite may be expected to adjust itself to *Fraxinus excelsior* in Europe and to *Fraxinus americana* in America; and that the earlier mycologists were often inclined to consider that a fungus must be a new species if it came from Asia or America, and many of these names that are really synonyms are still in current use. It is especially to be noted that those who study the fungi in both Europe and North America find great numbers of species indistinguishable on the two continents. Mycologists can attest to many striking cases of the wide distribution of species of fungi. Spores of fungi can travel faster and farther than can seeds, although they can scarcely cross an ocean unless aided by man. Land bridges in past geologic ages are considered to have allowed plants to cross the area that is now the North Atlantic; seed plants on both sides are still similar, though often specifically distinct; the fungi are likewise similar, and often unquestionably identical.

Reichert (15) in 1921 made a careful analysis of the fungus-flora of Egypt, from which the following figures are taken: 23 per cent of the 237 species then known from Egypt were cosmopolitan; another 27 per cent had a wide distribution; about 21 per cent were more restricted to Mediterranean regions; and nearly 29 per cent he found to be endemic, whereas only 107 species (7 per cent) of the 1503 Egyptian phanerogams were stated to be endemic. Reichert's data agree in trend with those we have assembled, except for the high percentage of endemism reported for the Egyptian fungi. Reichert records 68 species, some 40 of which he himself described as new, as endemic to Egypt. There can be no doubt, however, that many of these species occur outside of Egypt. In Manitoba (2) 45 species, or 2 per cent of the fungi, were found to be new species, and temporarily had to be considered endemic, whereas only one seed plant (*Gentiana ventricosa*) is thought to be endemic to Manitoba, and it is a doubtful species. As a matter of fact, there are perhaps really no endemic phanerogams or fungi in Manitoba: some of the new species of fungi first found there are already known to occur more than a thousand miles east or west. It is perhaps worth while to supplement Reichert's data for Egypt with an analysis made from Melchers' (11) recent paper. Melchers records 354 species of fungi as now known in Egypt: 29.4 per cent of these fungi apparently occur also in Manitoba. Many of the fungi common to the two areas are, of course, parasites of cultivated plants; yet only about 19.9 per cent of the species of hosts listed by Melchers occur cultivated or wild in Manitoba. The fungi of Egypt

25

evidently have a wider average distribution over the world than the phanerogams which grow in Egypt.

A comparison of the classes of fungi in respect to their distribution naturally shows considerable variation. The Myxomycetes have usually a very wide distribution, as amply recorded in the Listers' Monographs, and as evidenced by the recent paper of Peck and Gilbert (12). The slime moulds utilize types of substrata that are almost ubiquitous, and they are not conspicuously affected by climatic factors.

Many of the Phycomycetes have also a wide distribution. About 85 per cent of the known Phycomycetes of Manitoba are also in Europe, and some 40 per cent of them also in India. Many of the Peronosporales, on the other hand, have a distribution limited by that of their hosts.

Amongst the Ascomycetes, species of Pezizales have a range that is frequently great. Seaver's book (18) on the operculate Pezizales has been checked as to the records of distribution, and it is found that only 35 per cent of the fungi included are known only in North America; 61 per cent include Europe in their range; the remaining 4 per cent have a distribution extending to Australia or other countries in addition to North America. As Seaver states: " Like other higher fungi, many of the cup-fungi are cosmopolitan or widely distributed over the surface of the earth, apparently having little regard for climate, altitude, or other factors which have such a powerful influence on the distribution of the higher plants."

The Pyrenomycetes have an average range somewhat more restricted than that of the Pezizales, because many of them are strictly limited to certain hosts. The Pyrenomycetes are inadequately known, but about half of the species which occur in Manitoba appear to be the same as species occurring in Europe. However, only about 12 per cent of the Pyrenomycetes known in India are considered identical with European species. Many Pyrenomycetes are known only from the tropics. The Erysiphaceae, as recorded in Salmon's Monograph (16), are rather well known, and have usually a wide distribution: about half the species and varieties listed for North America are known also in Europe.

The Ustilaginales are generally considered to show a wide distribution, particularly because the important cereal smuts are widespread. An analysis of the records in Clinton's work (6) shows that 114 (55 per cent) of the 206 species listed were known only from North America. Even when one excludes those found on cultivated plants, no more than about 60 per cent of the smuts of North America are endemic.

The distribution of the Uredinales is similar to that of the Ustilaginales. Both orders include parasites which are found in nature only upon vascular plants. About 60 per cent of the North American Uredinales of known life cycle are now considered endemic to this continent. Discussions of the distribution of the Uredinales have already been presented in some detail by several authors (13, 1, 5, etc.). At first thought it might seem that species

of rusts would have a distribution as restricted as that of species of the seed plants; but the majority of rusts can attack more than one species of host, and the potential range of a rust is the sum of the ranges of its hosts. Thus 27 per cent of the Indian rusts with known life cycle occur in Europe (5), and the rust flora of the plains of North America is strikingly similar to that of the steppes of eastern Russia and northwestern Siberia (1). Even obligate parasites appear to have a range averaging greater than that of phanerogams.

In respect to the Hymenomycetes, it is generally recognized that a large proportion of the Agaricales of North America are indistinguishable from species found in Europe. As Rea and Ramsbottom (14) state: "The majority of species observed [in the United States] were those we meet with in this country [England] and were typical in every way. Occasionally . . . the species was obviously the European species, but the range of variation was different." The relatively large size of many Hymenomycetes permits the bases of comparison with phanerogams to be similar. Burt has recently published a paper (4) on the hymenomycetous lignicolous fungi of the Siberian area. It is noteworthy that 44 per cent of the 127 fungi recorded occur also in Manitoba, which is about the same north latitude but removed approximately half the distance around the world. Of the 56 polypores given by Burt, 41 per cent are amongst the 98 species found in Manitoba. Forty-three per cent of the Thelephoraceae listed are common to Siberia and Manitoba, and Burt has made the determination from both countries. Now in comparing the hosts of these Asian fungi, it is found that of the 33 woody host plants specifically recorded, only one species (*Juniperus communis*) occurs also in Manitoba. In other words, there are 3 per cent of common species of phanerogamous hosts as compared with 44 per cent of common species of fungi. It is true that 29 of the 33 phanerogams are of the same genus with plants occurring in Manitoba; but there are apparently definite specific differences between the seed plants of northeastern Asia and those of central North America, whereas the accompanying Hymenomycetes, many of which are not highly specialized to specific hosts, are much more often specifically indistinguishable.

The Gasteromycetes are saprophytes, many of which are widely distributed, although certain species—notably in the Phallales—are tropical. The Fungi Imperfecti have, of course, distributions like the Ascomycetes. Blochwitz (3) has discussed the habitat and distribution of various moulds.

The soil fungi are somewhat heterogeneous, but the Moniliales and Phycomycetes predominate. An analysis has been made of the distribution records in the summary of the soil fungi by Gilman and Abbott (8). Of the 197 species of soil fungi recorded from North America, 34.5 per cent are known from Europe and 6 per cent from Asia. Of the 112 species reported by Gilman and Abbott as occurring in Europe, only 41 species, or 37 per cent, are considered endemic to Europe. There are 35 Phycomycetes

listed from soil in North America, and 26 of these, or 74 per cent, are also European.

Among the groups of fungi not already mentioned, aquatic fungi, coprophilous fungi, those causing fermentations, and many others are very widespread. Certain entomogenous fungi have a wide range, although the Laboulbeniales seem at present to have a more limited distribution.

The data presented above have indicated a wide average distribution for the fungi. There are, of course, many species of fungi which have been found only in small areas and cases in which species of spermatophytes have a far wider distribution than some of their parasites or saprophytes. It is fully to be expected that certain fungi may have been left behind as the phanerogams travelled in past geologic ages; that others may have disappeared after arrival in new localities; and that still others may have been newly evolved and have not yet had time to seek out the more distant ranges of their hosts or substrata. The principles seem clear: fungi commonly have a wide distribution, and a taxonomist can no longer expect that because a fungus is new to North or South America or to Africa it must be new to science. Mycologists, as they come more adequately to survey their fields, must look with increasing suspicion upon the numerous species " known only from the type locality."

Many problems regarding the distribution of the fungi remain unsolved; sometimes the distribution is less than we expect. Why, for example, should such common and widespread fungi as *Scleroderma vulgare, Plectania (Sarcoscypha) coccinea, Collybia radicata, Lepiota procera, Clitocybe illudens,* and *Armillaria mucida* be absent from Manitoba? Doubtless in some cases a favorite phanerogam is absent. We need more data on the effect of climatic factors on the distribution of the fungi; phytopathologists have shown clearly that temperature, moisture, light, and other factors influence the prevalence, destructiveness, and even the distribution of the parasites. No doubt this is true of the saprophytes also. *Phytophthora infestans* and *Plasmopara viticola* have been found in Manitoba only near the end of the occasional succession of damp summers. *Amanita caesarea* for some reason does not extend far into the north; *Puccinia glumarum* remains in the west in North America. Climatic factors unquestionably influence, directly or indirectly, the distribution of many fungi, but the majority of fungi have a wide range despite climate.

The distribution of the angiosperms is said (9) to be primarily controlled by the distribution of climatic conditions, and only secondarily controlled by the distribution of edaphic factors. We must evidently reverse the order for controlling factors in the case of fungi and state that the distribution of the fungi is primarily controlled by the distribution of their hosts and substrata. Cryptogams other than fungi have also commonly a wide distribution: thus nearly 70 per cent of the native pteridophytes of Manitoba are native also to Europe.   .

SUMMARY

1. The number of species of fungi on earth is of the same order as the number of species of phanerogams, although in the present state of knowledge the latter outnumber the former by about two to one.

2. In any particular state or country, at least in temperate regions, the species of fungi can be expected to outnumber the species of spermatophytes.

3. The smaller the area surveyed, the more the species of fungi outnumber those of flowering plants.

4. The fungi are associated predominantly with the phanerogams.

5. The average distribution (range) of species of fungi is greater than the average distribution of species of phanerogams.

6. In general, saprophytes have wider distribution than parasites. Saprophytes, such as Myxomycetes, Mucorales, Pezizales, and Gasteromycetes, which are not commonly specific as to substrata, usually have the widest distribution; but even the obligate parasites, because they can commonly parasitize more than one species, often have a greater range than their individual hosts.

7. The distribution of hosts or substrata primarily controls the distribution of the fungi.

8. Climatic factors have less effect upon the distribution of the fungi than upon the distribution of seed plants.

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CYTOLOGICAL STUDIES ON THE PARASITIC RELATIONSHIP OF *UROCYSTIS CEPULAE* TO THE ONION

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The morphology of the onion smut fungus and its behavior during the various developmental stages of its life history in the host have been considered at one time or another since 1872 by a number of investigators. Ware (1869) first brought the disease to the attention of a Massachusetts agricultural society and pointed out the importance of the protracted persistence of the causal organism in infested soil. Walker and Jones (1921) dealt primarily with the relationship of soil temperature to onion smut infection. Anderson (1921) made a study of the saprophytic mycelium in culture, as well as detailed observations upon the behavior of the organism in the susceptible host. Blizzard (1926) also studied the fungus in culture and, furthermore, followed the nuclear behavior both outside and within the host plant. There are certain points in the life cycle of *Urocystis Cepulae* Frost at which further evidence is necessary, either to fill in gaps or to lend support to information already reported. The present work was begun with this purpose in mind, and although cultural and other preliminary studies upon the saprophytic mycelium were made, efforts were finally centered particularly upon the attempt (1) to determine if possible the exact mode of penetration of the parasite into the young, susceptible host, and (2) to discover cytological evidence for whatever changes might occur in the host, in the parasite, or in both organisms with the establishment of immunity by the onion seedlings.

MATERIALS AND METHODS

In order to obtain young infected seedlings for the purpose of studying early penetration stages, two methods were employed. At first soil from the Racine, Wisconsin, onion-growing region was used without a great deal of success. This soil was used because onion smut was known to be prevalent in that region. Southport Red Globe onion seed was planted in flats of the Racine soil. These flats were then placed in a cool greenhouse. The temperature here remained almost constantly at approximately 65°F. while the seeds were germinating and during early stages in the development of the seedlings. An abundance of infection was readily secured, but very early penetration stages were not found.

A second method proved more successful. Diseased seedlings which had been grown in smut-infested soil were pulled up and the cotyledons bearing lesions containing ripe spores were removed. Portions of these

diseased cotyledons were surface-sterilized in a 1 : 1000 mercuric chloride solution, washed in sterile distilled water, and smeared upon onion-extract agar in sterile petri dishes. After the spores had germinated, fairly well-isolated individuals were marked and allowed to grow until masses of mycelium about 2.5 mm. in diameter were produced. At this time sterile onion seedlings four days old were placed in contact with the growth of fungus in the petri dishes. After periods of two, three, and four days, respectively, they were killed and fixed in separate lots. The onion seeds from which these seedlings were grown were soaked in distilled water over night, then placed in a 1 : 1000 mercuric chloride solution for three minutes in a partial vacuum in order to wet the entire seed coats, which were infolded and wrinkled. The seeds were then washed in sterile distilled water and placed in sterile petri dishes on moist filter paper to germinate.

Most of the commoner killing and fixing agents were tried at room temperature with indifferent or poor results. At the suggestion of Dr. J. G. Dickson, the temperature of two of the fixing solutions which had shown the most promise at room temperature was raised to 85°C., a point well above that at which coagulation of protoplasm occurs. Because of the relatively thick layer of cutin which is present outside the very delicate, succulent tissue of the young onion seedling, penetration of the fixatives at ordinary temperatures was too slow to prevent a great deal of plasmolysis and even violent distortion of tissues. By increasing the temperature of the fixatives, these difficulties were avoided to a considerable degree. No doubt most of the solutions tried at room temperature would have behaved in a more or less satisfactory manner if higher temperatures had been used.

One or the other of the following killing and fixing solutions was found to be satisfactory for seedlings of this age:

(1) One-fourth Flemming's weak solution and three-fourths distilled water plus one per cent urea.

(2) Allen's modification of Bouin's picric acid fixative.

The best results were obtained by plunging the material to be fixed into one of these fixatives, heated to 85°C., and then fixing for 12 hours. Sections were cut from 8 to 12 μ thick. The stains tried were Pianeze IIIB, Heidenhain's haematoxylin and erythrosin, Delafield's haematoxylin and Orange G, and safranin and fast green. The use of a combination of safranin and fast green was found to be the most satisfactory method of staining the parasite within the host tissue. The host tissues were stained varying depths of green; the nucleoles of the host nuclei were red. The fungus varied in its ability to retain the stains. In a healthy condition the mycelium stained with both safranin and fast green. As degeneration set in, the dying mycelium stained definitely with safranin, assuming a dull red color. With further degeneration, the ability of the fungus to hold safranin was lost, and finally the walls of definitely dead and empty strands of mycelium stained only with the fast green. As long as the nuclei of the parasite were present, they could usually be detected by means of their red nucleoles.

For the purpose of studying the seedlings as they were passing from the early susceptible stage to the later immune stage, healthy seedlings were grown in muck soil free from onion smut spores. After many of the seedlings were well above the surface of the soil, they were thinned out to leave an even stand. Plants from seeds which were delayed in germination were removed as they appeared. Approximately a week before the seedlings would have become immune, they were transplanted each day in lots of about forty into infested soil. The infested soil used was prepared by inoculating some of the same lot of aforementioned muck soil in the following manner. Badly smutted set-onions which had been collected at Racine, Wisconsin, were dried and pulverized. The resulting powder was well mixed with the soil in order to obtain as uniform a distribution of spores as possible. Half of each transplanting of seedlings was removed after six days' growth in the smut-infested soil and fixed. All the other transplanted seedlings were allowed to remain for 26 days, after which time they were removed and the diseased condition was noted. All the plants in the clean soil which were not used in the experiment were left as checks. At the end of a month the checks were all removed and carefully examined under a binocular dissecting microscope. In no case did the checks show evidence of disease. It was found even more important to kill and fix the older seedlings than the younger ones as nearly instantaneously as possible. In the older seedlings the cuticle of the cotyledon was somewhat thicker and the cells were even more vacuolate than in the seedlings six or seven days old. The dense cytoplasm was apparently spread in an extremely thin layer on the inside of cell walls. The walls, however, were not markedly thicker than those in the younger seedlings. In order to fix this succulent tissue, it is necessary that killing and fixing be accomplished as rapidly as possible in order to avoid complete collapse. The solution most successful as a killing and fixing agent for these older seedlings was found to be Allen's modification of Bouin's fluid heated to 85°C. The fixed material was embedded in paraffin and sections were cut from 9 to 15 microns in thickness and stained. A combination of safranin and fast green again gave the most satisfactory preparations.

OBSERVATIONS AND DISCUSSION

Early penetration stages

Farlow (1876) reported the fungus *Urocystis Cepulae* Frost as having been found in all the tissues of the onion seedling with the exception of the epidermis. Whitehead (1921) considered that infection invariably takes place within 2 mm. of the collar of the young seedling and frequently immediately at the collar. The "collar" refers to the transition region, approximately at the place where root and cotyledon are attached to the short, embryonic stem. The presence of a number of distinct lesions in a single cotyledon was explained by Whitehead by the assumption that the

sporogenous hyphae, which had spread throughout the cotyledon from the source of infection in the collar region, formed spores intermittently, leaving what seemed to be healthy tissue between the lesions. This process was called " discontinuous spore-formation." Apparently no attempt was made to determine whether or not strands of mycelium connected the lesions.

Anderson (1921), on the other hand, concluded that each separate lesion in a cotyledon is produced by a distinct infection hypha. In other words, the cotyledon while still below the surface of the soil may be attacked at practically any point along its length. This is in accord with my findings. Penetration, according to Anderson, may also take place from the cotyledonary cavity. This is entirely possible, since the slit in the wall of the cylindrical portion of the cotyledon, through which the first leaf emerges, appears very early. No instances of such a mode of infection were observed, however, in the course of the present study.

Very early penetration stages have never been figured nor described for the onion smut fungus. Figure 2 of plate 11 illustrates as early a stage of penetration as I have been able to find. The infection hypha has pierced the cuticle and has gone almost entirely through the sub-cuticular layer, but as yet has not actually penetrated into the interior of the epidermal cell. A somewhat later stage is shown in figure 3 of plate 11. Here the fungus has penetrated into the cell, but the opening through the cell wall does not appear because of the angle at which the hypha penetrated and that at which the section was cut.

In all cases observed, a space is found about the hypha at the point where it passes through the outer epidermal wall. Occasionally the hypha is constricted at this point (fig. 1, pl. 11), but ordinarily the infection hypha is of the same diameter where it passes through the wall as it is further along in the interior of the cell. The wall material beneath the cuticle is apparently acted upon by a fungous secretion, perhaps enzymatic, produced by the infection hypha, which seems to aid the fungus in dissolving its way through the relatively thick layer of the outer wall. Judging from the single example seen (fig. 2, pl. 11), a certain amount of pressure is exerted by the hyphal tip, which apparently has been able noticeably to bulge the innermost layers of the wall. In no case, however, was there any evidence of any sort of anchorage or anchoring structures such as an appressorium which might aid the penetrating hypha in its passage through the cuticle. In fact, as Miss Pearson (1931) observed in her studies of *Gibberella saubinetii*, the strand of mycelium whose growing end has already entered the host is almost always separated from the cuticle by a space. No sort of attachment to or connection between the cuticle and the portion of the penetrating hypha outside the onion seedling was observed. However, hyphae have never been found which were just in the act of establishing relations with the host. Further, that portion of the hypha which remains outside the host for a time degenerates and crumples very soon after penetration has taken place (fig.

1, 2, 6, pl. 11). Consequently it would be difficult to say with certainty that the growing hyphal tip had not been attached to the host by some means at the point at which it was about to enter.

After the initial infection hypha has penetrated the cell wall, it branches readily, and its branches sometimes, but by no means always, grow toward and more or less enfold the host nucleus. A clear canal with tapering, funnel-shaped walls has been reported (Anderson, 1921) as leading into the epidermal cell from the outside. The nature of this funnel-shaped structure was not determined. Apparently it was an inward-growing sheath of wall material, since it seemed to be continuous with the outer epidermal wall. It was also suggested as possible that the wall of the infection hypha might thicken and take part in the formation of this tube or canal.

Brefeld (1895), in his studies of the penetration of oat seedlings by the conidia of *Ustilago levis*, found that funnel-shaped structures apparently were produced at the point of entry by the swelling of the " membranes of the penetrating tube." Several figures illustrating these structures were given. Lutman (1910), working later with the same form, described a process of stimulated wall-formation on the part of the host at the point of infection. As layers of wall material are deposited toward the inside, the invading hypha dissolves its way through them until finally, when entrance into the cell is effected, a funnel-shaped tube of host wall material is found to be present about the infection hypha.

Although no such definite structures have been found in this study at the point of infection, it is quite common to find what appears to be a closely investing sheath of host cytoplasm surrounding the infection hypha (fig. 1, 3, pl. 11). As far as could be determined, this cytoplasmic sheath is present about the infection hypha only for a short distance from the point of entrance into the host cell. Extensions of the original invading strand of mycelium twist about in the vacuole of the host cell without any apparent ill effect upon the cell. In none of the preparations showing penetration stages did it appear as though either the nuclei or the cytoplasm of the invaded onion cells were responding in any way whatsoever to the presence of the fungus. The staining reactions of parts of parasitized cells are, microscopically at least, similar to those of uninvaded cells. So far as could be determined, there is no rearrangement or diminution in size or amount of the nuclei or cytoplasm of invaded cells. At the time penetration stages are found most abundantly, the epidermal cells, particularly, are highly vacuolate, with only an extremely thin layer of cytoplasm lining the cell wall. Occasionally, however, the invaded cell appeared to be a trifle swollen or perhaps a little larger than the adjoining cells. As no differences between invaded and uninvaded cells can be observed as to the reaction to the killing and fixing solutions, it would be difficult to determine microscopically whether or not differences in turgor exist. It is entirely possible that the invaded cells in question simply happened to be larger than neighboring cells and bulged a trifle above the general level.

All these observations seem to point to the fact that the fungus, at least in its early relations with the host, causes little disturbance in the functioning of the host cells.

Haustoria, as such, have never been observed.

Studies of seedlings passing from susceptibility to immunity

It has been quite definitely established (Walker and Jones, 1921; Anderson, 1921) that immunity of the tissues of the onion cotyledon to invasion by the onion smut fungus is coincident with the maturing of such tissues. Under greenhouse conditions such a state of immunity is reached ordinarily between the nineteenth and twenty-fourth day after sowing of the seed—at approximately the time that the first true leaf emerges from the cotyledon. In order to determine with certainty whether maturity of the cotyledonary tissue is responsible for the immunity of the seedlings, a number of seedlings which had passed the susceptible stage were carefully stripped of their cotyledons and the immature tissue beneath was exposed to the smut-infested soil. Sixty per cent of such plants were reported to become diseased (Walker and Jones, 1921).

The seedlings which were to be used for a cytological study of the host-parasite relationship as the young onion plants approached immunity were grown, as has been said, in smut-free soil and transplanted into smutted soil. As the seedlings grew very rapidly in the clean soil, transplanting was started on the sixteenth day after sowing and further transplantings were made on succeeding days until the first foliage leaves began to appear from the cotyledonary openings. Other similar series were grown and material was collected from them, but since there were no significant differences between series, only these six transplantings of the first series will be referred to hereafter as lots 1 to 6, respectively.

It will be remembered that at the end of six days' growth in the smutted soil, half of each transplanting was removed and fixed. The other half was allowed to remain for almost a month, at which time the seedlings were removed and examined for signs of disease. These diseased checks, corresponding to fixation lots 1 and 2, showed 40 and 67 per cent disease, respectively, at the end of this period of growth in the smutted soil. The lesions were relatively large and, because of the masses of spores present, were typically black. Bending of the seedlings occurred at the points where lesions were present, the masses of spores characteristically being in the convex, bulging portion of the diseased tissue. All lesions were, of course, found in the lowermost centimeter and a half of the cotyledon, since that part of the transplanted seedling was all that had been in contact with the smutted soil. These symptoms are comparable to those occurring in normally infected cotyledons. In diseased check lots 3, 4, and 5, although the amount of disease ranged from 71 per cent in lot 3 to 43 per cent in lot 5, the lesions became progressively less noticeable. In fact, in lot 5 the lesions were

almost thread-like and barely visible. Instead of being black, the color of the diseased areas was grayish because of the smaller numbers of spores present. Neither bending nor twisting of the plants was apparent. Attention may be called to the fact that, although the actual percentage of diseased plants seemingly did not lessen as immunity was approached, the type of lesion became markedly different and the severity of the disease was much reduced. In lot 6, out of seventeen seedlings examined only one was slightly diseased. This seedling had apparently come from a seed whose germination had been delayed. When examination of the other seedlings was made, all of them had already put out the second foliage leaves and in some the third leaves had appeared, whereas, in the single diseased individual of lot 6, only the first leaf was beginning to show.

Cytological study was begun with the material fixed from lot 5. It seemed from an examination of the diseased checks corresponding to this fixation and to the next, lot 6, that the factor or factors playing a part in the actual inhibition of the entrance of the organism into the host, in an unfavorable reception of the fungus by the content of the host cells, or at least in the bringing about of some sort of conditions leading to an unsatisfactory host-parasite relationship, would be most evident at this particular stage.

It was found that apparently normal mycelium was often present in the seedlings of lot 5. This healthy mycelium, however, was never observed to have penetrated very deeply into the host tissue. The mycelium found most frequently in lots 5 and 6 was ordinarily present in epidermal or sub-epidermal cells. According to Anderson (1921), after five or six days' exposure to smutted soil, definitely susceptible seedlings should at least begin to show evidences of spore-formation, providing, of course, that penetration occurred shortly after exposure. Quite commonly the hyphae which were found in the epidermal cells and occasionally in the sub-epidermal cells were characteristically contorted, misshapen, or irregularly swollen (fig. 10, pl. 11; fig. 11, 15, 16, pl. 12). The mycelium in question, as compared with healthy mycelium, stained very differently. Young, healthy strands of hyphae which are present in epidermal cells, or which are just becoming intercellular, stain as follows with a combination of fast green and safranin: The cell walls are green, nucleoles a bright red, and the more or less homogeneous cytoplasm, apparently having an affinity for both stains, assumes a dull purplish appearance. If degeneration of the mycelium in the epidermal cells of the seedlings passing from susceptibility to immunity has not proceeded too far, the hyphae stain deeply with safranin only. The cytoplasm in these hyphae becomes more or less granular and finely vacuolate. After degeneration and death have occurred, the empty cell walls of the dead hyphae stain green.

It is true that mycelium in the epidermal cells of susceptible seedlings dies very soon after the intercellular mycelium has become well established between the deeper-lying cells of the cotyledon. Although this epidermal mycelium is somewhat swollen as compared with the more uniform inter-

cellular hyphae, it is readily distinguishable from the coiled and contorted strands present in the epidermal cells of the immune or nearly immune host. Ward (1902), Miss Marryat (1907–1908), Stakman (1915), and Miss Allen (1926), in studies with hosts partially or highly resistant to grain rusts, particularly *Puccinia graminis tritici*, found that either invaded host cells or cells in contact with invading hyphae sooner or later suffered from the presence of the parasite because, possibly, of the excretion of certain toxins, and eventually underwent marked changes which led to death. So far as could be determined, as has been already suggested, no harmful effects to host cells followed the invasion or establishment of the onion smut fungus in either susceptible or nearly immune onion seedlings. In fact, it is possible to find, in invaded cells of plants of lots 5 and 6, mycelium which is badly degenerated or already dead and host cell contents which are still apparently healthy, so far as may be judged from general appearance and staining reactions.

It was early noted in the cytological study of the hosts in lots 5 and 6 that a peculiar dichotomous forking of the fungal branch tips was in evidence in the epidermal cells (fig. 11, 12, 14, 15, pl. 12). This behavior of the parasite at this particular stage seems to be characteristic. Branches which occasionally proceed from the epidermal mycelium and penetrate sub-epidermal cells are almost invariably branched once or twice dichotomously (fig. 12, 13, 16, pl. 12) and are similar in appearance to the haustoria described by Anderson (1921) which, he states, "are always very much branched, but the branches may be reduced to mere knobs or short stubs which are frequently bifid at the apices. . . . These absorbing organs are not numerous, but are not uncommon." Anderson did not indicate specifically the age of the host material in which these haustoria were found, and it is possible that seedlings were chosen which were just passing into the immune stage. He does, however, figure at least two examples of haustoria entering cells from apparently healthy intercellular strands of mycelium. In the present study these abortive branches were carefully observed in the older material. In no case were they found to arise from mycelium which occasionally has been able to escape from epidermal or sub-epidermal cells and become healthy, intercellular mycelium.

It seems to be evident that the contorted mycelium present in the epidermal cells and the abortive, haustorium-like branches which the fungus produces in these cells themselves or which it is able to push out into the sub-epidermal cells indicate a response on the part of the parasite to an unfavorable environment. It is possible that some toxic substance or substances which may or may not have been present in harmlessly small amounts in the young, susceptible seedlings may have either made their appearance or increased to such an extent with the maturing of the tissues in the seedlings passing into the stage of immunity that growth of the parasite is markedly inhibited after its entrance into the host. As evidences are found of renewed

growth and of the production of healthy intercellular mycelium from epidermal mycelium that apparently, from microscopic indications, was earlier inhibited in its growth, the behavior of the onion smut fungus in its host-parasite relationships may be at this time at least somewhat comparable to that of *Puccinia glumarum* in the tissue of American Club, a wheat which is, according to Marryat (1907), moderately resistant to the attack of the yellow-rust organism. She reported that " the invading fungus is by no means flourishing in American Club, but though baulked at one point it manages to hold its own at another, until it finally succeeds in forming a moderate number of small, scattered pustules. . . . Examined in section, however, only a relatively small number of these are found to produce normal spores which succeed in bursting the epidermis. The remainder exhibit for the most part all stages of abortion. . . ." Another instance may be cited. Kanred, a wheat immune to many strains of the stem-rust organism, is subject to attack by certain strains which can produce pustules. According to Allen (1923), " A fungus which has been checked in its first attempt to establish parasitic relations with the host may still possess enough vigor to grow. . . . An extreme case of this sort is seen where a fungus made no less than six distinct attacks. . . . In some cases the reaction between host and parasite is more sluggish than in the examples described. The fungus makes a full-grown haustorium and gains enough food from it to enable the hypha to branch and grow on to the next cell before the first haustorium and the invaded host cell die. The new hypha makes a second haustorium and this may be repeated several times and further branching may occur. The interaction of host and fungus is slower in starting and not quite so severe in its effects. The result is a succession of dead, discolored hyphae and dead host cells and an ever weakening advance of the fungus."

Structures which will be referred to as sub-cuticular vesicles are found in this older material, particularly in lot 6. They appeared so consistently in sections made from seedlings which were passing from susceptibility to immunity that it was thought that they might possibly represent an effort on the part of the host to exclude, at least partially, the entrance of the fungus.

A typical vesicle is shown in figure 17 of plate 12. It will be seen that the content of this vesicle has apparently digested a place for itself in the sub-cuticular material of the cell wall. With the idea in mind that these small masses of material might be due to irregular depositions of cutin, slides were stained with Sudan III. The content of the vesicles did not retain the stain, this fact probably indicating that the material was other than cutin. Cotton blue, however, was retained by this material, while it bleached out of all the host tissues readily when absolute alcohol was used to wash the slides. The retention of the cotton blue by this material probably indicates that it is of fungous nature. An effort was next made to discover, if possible, whether or not the imprisoned mass of " fungus," if that is what it is, is ever able to penetrate from the vesicle through the cellulose wall into the epidermal cell.

Only two examples were found which were at all indicative of such actual penetration (fig. 18, 19, pl. 12).

No connection of the content of any of these vesicles with mycelium outside the host has yet been found. The portion of the infection hypha which remains outside the epidermal cell ordinarily crumples and disappears shortly after infection. Consequently, it is not impossible to conceive of the external portion of the infection hypha as having been dislodged during the processes preceding sectioning, particularly if it were perhaps constricted at the point where it entered the host. It would seem, however, that evidence of an opening through the cuticle might occasionally have been found. The idea that these vesicles were connected with immunity was based almost entirely upon the constancy of their presence in the host material which had just reached a state of immunity, as evidenced by an examination of disease checks. Vesicles were never found in the walls of epidermal cells from material of the same age grown in soil taken from the same source as the artificially inoculated soil. Figure 8 of plate 11 shows the wall of an epidermal cell at the edge of the section, in surface view. Apparently the mycelium has made its way beneath the cuticle and has remained there and grown somewhat for a time, dissolving what appears to be a clear region in the sub-cuticular layer. Figure 9 is from the next serial section. This represents a view of the interior of the same cell and shows a mass of markedly inhibited mycelium which appears to be dead. It seems at least probable that this condition last referred to may represent a step between the condition of a vesicle containing what appears to be completely inhibited mycelium and that of mycelium which enters the seedling as readily and directly as it does in the early, susceptible stages but soon responds unfavorably to an unsuitable environment—an environment which has, however, not yet become so completely unsuitable as to preclude entrance entirely, or to cause this entrance to be quite irregular.

No reference in the literature has been found to a comparable behavior on the part of any other parasitic fungus.

Considering the earlier fixations (lots 1 and 2), it was found after sectioning and staining that the mode of penetration was normal and that uniform, intercellular mycelium was present in abundance deep in the cotyledonary tissues. Indications were found of the massing of mycelium preparatory to spore-formation.

In lot 3 almost all of the mycelium present had early become intercellular. An infected epidermal cell was occasionally observed in which the fungus was apparently in a not quite favorable environment. While some of the branches might grow out from such an epidermal cell and become intercellular, one or more of the other branches would be somewhat distorted, stain heavily with safranin, and possess dichotomously branching tips. This irregular behavior seems to point toward the beginning of an unfavorable host-parasite relationship.

In lot 4 distorted, degenerating mycelium was more common than in the preceding fixations. A few sub-cuticular vesicles were also found in the walls of the epidermal cells of these seedlings. Although here an abundance of intercellular mycelium is still present, none of it has penetrated into the tissues of the cotyledons deeper than the third sub-epidermal layer of cells. Much of this mycelium is apparently healthy, but the ends of some of the branches are swollen. Branches of the initial growth of the fungus in the epidermal cells which are about to grow out from or have grown out from these cells are often found to be somewhat swollen at the points where they are in contact with the cell walls, producing structures rather appressorium-like in appearance. Some of these irregular structures are almost similar to the occasional swollen tips of definitely degenerating mycelium in the epidermal cells of lot 5 (fig. 16, pl. 12), and may represent an intermediate condition between mycelium which is able to grow out from the epidermal cells of the susceptible host readily and the hyphae in the immune or almost immune host which are often completely retained within the epidermal or sub-epidermal cells until degeneration of the fungus is complete.

Much of the intercellular mycelium in lot 4 appears as if it were preparing for sporogenesis, even though it very seldom penetrates farther into the host than the third sub-epidermal layer of cells, whereas mycelium which has penetrated a definitely susceptible host ordinarily occupies a more or less central position in the cotyledon when it is massed for chlamydospore formation. The hyphae in lot 4 are much more loosely arranged than they are in the masses of mycelium which are ordinarily aggregated preparatory to spore-formation in the susceptible seedling. Swollen, rounded fungous cells are often present which are definitely binucleate and which appear as though they might be young abortive spores, since they are without the usual close investiture of accessory cells. Apparently normal spores are sometimes very sparsely produced, however, as has been shown by an examination of the diseased checks corresponding to lot 4.

According to results obtained cytologically, it appears that the factors to which the development of immunity by the onion seedling are due become, at least under greenhouse conditions, more and more pronounced over a period of approximately four days until the host reaches such a state that it is able to combat successfully the activity of any mycelium which has been able to penetrate the epidermal cells. It is at this time that complete immunity is attained by the onion seedling.

SUMMARY

1. No indications of definite attaching structures, such as appressoria, or of any sort of anchorage of the fungus to the epidermis of the host were found in the material studied.

2. Examples of early penetration stages indicate that both mechanical pressure and enzymatic action may be concerned in the entrance of the fungus into the host through the epidermal cell walls beneath the cuticle.

3. No cytological nor morphological changes were noted in the invaded epidermal or sub-epidermal cells of the host.

4. Haustoria were not observed.

5. As a condition of immunity is approached with the maturing of the tissues of the cotyledon, the lesions resulting from infections occurring during this time of approach toward the establishment of a condition of immunity become less and less noticeable instead of becoming fewer in number.

6. Microscopical examinations of a series of seedlings fixed at 24-hour intervals at a time when the plants were passing through transition stages between susceptibility and complete immunity indicate that certain changes become more obvious in the appearance and behavior of the parasite the nearer a condition of immunity is approached. At this time the hyphae which have been able to make their way into the epidermal cells of the host become progressively more and more distorted and begin to show signs of degeneration. Ultimately, when immunity is attained, degeneration of the fungous strands is almost complete.

7. Structures referred to as sub-cuticular vesicles were found consistently in onion tissue which was just becoming immune. These structures perhaps indicate a more or less successful attempt on the part of the host to inhibit completely the passage of invading fungi beyond the innermost layer of the outer wall of the epidermal cell.

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EXPLANATION OF PLATES

All drawings were made with a camera lucida at table level. Leitz 15 × oculars and a 2-mm. oil-immersion apochromatic objective were used. Magnification in all cases is about 2230 ×. The drawings have been reduced one-half in reproduction.

PLATE II

Fig. 1. Penetration hypha, showing aperture through which the fungus penetrated and the disintegrated portion of the infection hypha which remains outside the host.

Fig. 2. Early penetration stage showing the result of the apparent dissolving action of the hyphal tip upon material of the sub-cuticular layer of the epidermal cell wall.

Fig. 3. Slightly later penetration stage. The infection hypha has become septate.

Fig. 4. Appearance of a strand of intercellular mycelium in cross section.

Fig. 5. Hypha which has grown from an epidermal into a sub-epidermal cell. No great amount of dissolution of wall material is apparent at this point.

Fig. 6. Healthy epidermal mycelium which is becoming intercellular. The older parts are dead and empty of cell contents.

Fig. 7. Sub-epidermal cell with mycelium which has penetrated from an epidermal cell and has branched profusely before finally becoming intercellular.

Fig. 8. Surface view of an epidermal cell wall from an onion seedling which is of such an age that it is just becoming immune to attack by the fungus. The infection hypha has made its way beneath the cuticle and there apparently has grown for a time, dissolving the sub-cuticular wall material before penetrating the interior of the cell.

Fig. 9. Interior view of the cell illustrated in fig. 1. The contorted mycelium is dead and devoid of cell contents.

Fig. 10. Epidermal cell from seedling fixed approximately one day before immunity was attained. The tips of the hyphae are swollen and contorted with granular, vacuolate cell contents.

PLATE 12

Fig. 11. Inhibited, dichotomously branched mycelium in an epidermal cell of a seedling approaching the condition of immunity.

Fig. 12. Epidermal cell from a seedling of the same age as the one illustrated in fig. 1. Degenerating mycelium with a dichotomously branched extension into a sub-epidermal cell.

Fig. 13–16 were drawn from preparations made from onion seedlings fixed at an age when they were closely approaching a condition of immunity.

Fig. 13. Dichotomously branched extension from an epidermal mycelium into a sub-epidermal cell.

Fig. 14. Dichotomously branched extension of the mycelium in an epidermal cell.

Fig. 15. Degenerating epidermal mycelium.

Fig. 16. Dead and dying contorted epidermal mycelium.

Fig. 17–19 were drawn from preparations made from onion seedlings fixed approximately at the time immunity was attained, as indicated by the examination of analogous check seedlings which were allowed to grow until a time at which disease would have been apparent.

Fig. 17. Sub-cuticular vesicle containing "mycelium."

Fig. 18. Vesicle containing "mycelium" which apparently has made an attempt to establish itself in the epidermal cell.

Fig. 19. Vesicle containing "mycelium" which has successfully extended a branch into the epidermal cell. The extension, however, is seemingly dead and devoid of cell contents.
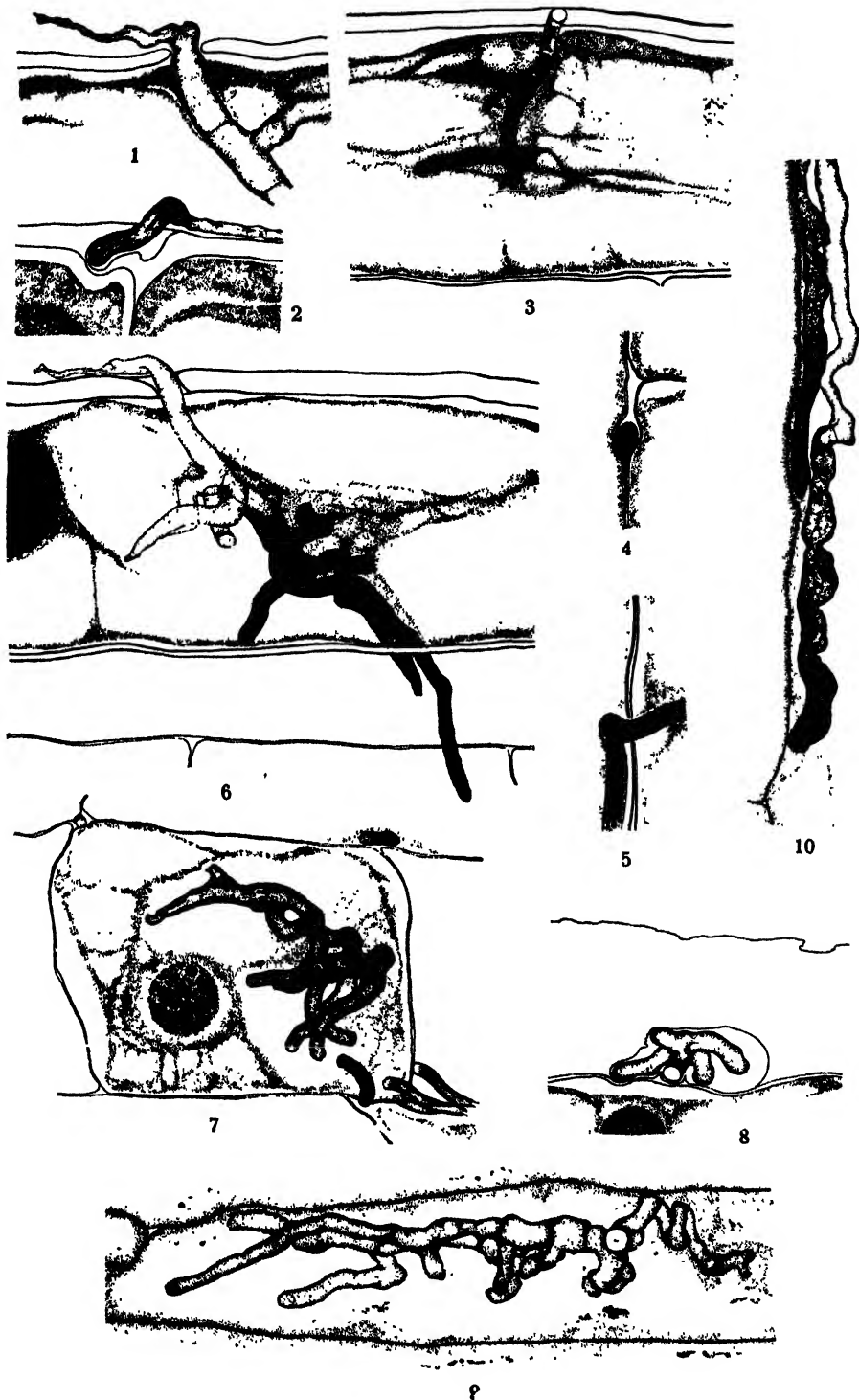
EVANS: UROCYSTIS CEPULAE

THE BEHAVIOR OF A TRIPLOID IN *NICOTIANA TABACUM* L.

E. M. EAST

INTRODUCTION

Triploidy was discovered by Gates (1908) in *Ocnothcra*. A little later it was reported and studied in similar ·material by Stomps (1910, 1912), Geerts (1911), and Lutz (1912). It was also noted in *Morus* by Tahara (1910) about the same time.

The majority of students believe that triploidy originates through the union of haploid and diploid gametes, though Gates (1929) thinks that this method—in *Ocnothcra* at least—is unusual and that dispermy is the usual cause.

One may distinguish between cases of true triploidy, where three similar genoms are involved, and cases of hybrid triploidy, such as that first described by Rosenberg (1909) in *Droscra*, where the mating is between two species forming gametes having chromosome numbers that may be denoted by the terms $2N$ and N, respectively. Only cases belonging to the first category (including here the mutants of De Vries) will be considered in this paper, though of course it is realized that the distinction is arbitrary.

Studies of triploid types have been made the basis of many papers. The contributions have involved three main points: the association of the chromosomes at meiosis, the distribution of the chromosomes at reduction, and the chromosome relations of the functional gametes formed.

The association of chromosomes at meiosis

In what was probably the earliest contribution on triploidy, Gates (1908) examined material from a hybrid purporting to be *Ocnothcra lata* × *O. Lamarckiana*. The plant had 21 chromosomes, however, and this led the author to suppose that it was really the result of a cross between *O. lata* and *O. gigas*. As almost invariably 10 chromosomes went to one pole and 11 chromosomes went to the other pole at the heterotypic mitosis of the PMC,[1] Gates assumed that non-homologous chromosomes paired. In a later paper (Gates, 1909) these conclusions were confirmed, except that it was noted that cells were sometimes formed having 9 or 12 chromosomal aggregates at IM. Geerts (1911), on the other hand, found that his hybrids between

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[1] The following symbols are used: PMC, pollen mother cell; IM, first metaphase; IIM, second metaphase: IA, first anaphase; IIA, second anaphase; IT, first telophase; IIT, second telophase.

the tetraploid *O. gigas* and the diploid *O. Lamarckiana* followed the *Drosera* system of pairing, the univalents commonly separating 4 and 3. The discrepancies between the observations of Gates and Geerts may have been due to differences inherent in the material, since the Oenotheras are notable for the complexities shown in their cytology. They may also have been due to temperature differences at the time of fixation. The material used by Geerts was fixed in September and October (see Lutz, 1912), and I have noticed in certain *Nicotiana* hybrids that low temperatures prevent pairing. At any rate, after the smear technique came into use, *Oenothera* workers found that trivalent associations frequently occurred (see Catcheside, 1931, and the literature cited there).

Adequate study of the association of chromosomes of triploids at meiosis began with a paper by Belling in 1921. From the investigations published since that date, one gathers that the situation is somewhat as follows: In *Canna* (Belling, 1921, 1925), in *Datura* (Belling, 1921, 1925, 1927; Belling and Blakeslee, 1922, 1923, 1924, 1926), in *Hemerocallis* (Belling, 1925), and in *Hyacinthus* (Belling, 1921, 1923, 1925, 1929; Darlington, 1929) the formation of complete sets of trivalents is so common that it may be called the characteristic method of association. In *Lycopersicum* (Lesley, 1926) and in *Zea* (McClintock, 1929) there is some irregularity shown, but trivalents are usually formed. In *Morus* (Tahara, 1910; Osawa, 1920), in *Campanula* (Gairdner, 1926), in *Prunus* (Darlington, 1928), in *Pyrus* (Darlington and Moffett, 1930; Moffett, 1931), in *Tradescantia virginiana* (Darlington, 1929), in *Petunia* (Dermen, 1930), in *Nicotiana* (Goodspeed, 1930), and in certain species of *Cotoneaster* and *Cratægus* (Moffett, 1931) there is great irregularity, with varying numbers of univalents and bivalents, together with an occasional trivalent.

In *Tulipa* Newton and Darlington (1929) and Darlington (1929) found that trivalent association was correlated directly with chromosome size. Darlington takes this fact to be an argument in favor of his theory that chromosome associations at meiosis are due to chiasmata.

It is to be noted that trivalents are not the most complex form of association found. A number of authors have observed groups of four or more chromosomes. In *Pyrus*, for example, Darlington and Moffett (1930) find a maximum association of nine chromosomes in the triploids, corresponding to a maximum association of six in the diploids. Analysis of these various associations leads the authors to believe that among the thirty-four chromosomes of a diploid *Pyrus* four chromosomes are represented four times and three chromosomes are represented six times.

The type of configuration which the chromosomes of triploids take during, or immediately preceding, IM has been studied intensively, particularly by Belling and by Darlington. According to Belling (1927), the short chromosomes with median constrictions, such as those of *Canna* and *Datura*, form all the possible configurations for end-to-end union of homologues

having attractive powers only at given ends. The short chromosomes with subterminal constrictions form more complicated configurations—as in *Hyacinthus* and *Hemerocallis*—since the homologues may be connected either at the ends or interstitially. Long chromosomes having median constrictions, as in *Hyacinthus,* have been seen as ring and rod and as chain, with or without other connection at the constrictions.

Belling thinks that there are five possible configurations for triploid association: the triple arc, the double arc and rod, the triple rod united at one end, the chain forming a V with unequal arms, and the chain that is more or less straight. He finds that the V is the commonest form and the triple arc is the rarest. Cytologists commonly agree on these configurations, though there is some dispute as to whether each type is distinctive.

From such cytological evidence, and from the results of a few relevant pedigree-culture experiments, geneticists have come to a general agreement that the ideal state in triploids is essentially the same as that found in diploids during the early stages of meiosis. This situation is a gene-by-gene association among chromosomes that have a gene-by-gene homology. For these reasons, it is not illogical to assume, as do various workers, that the comparatively low frequency with which N trivalents are found at IM in triploids is due to the disruption of associations that had existed at synizesis. Nor is it illogical to interpret the pairing sometimes found in haploids (Collins and Mann, 1923) as being the result of polyploidy, or to assume (Darlington and Moffett, 1930) that where multivalent associations exist, multivalent homologues are indicated.

Gene-by-gene association of homologues, where such association is possible, therefore, is a device demanded by the pedigree-culture evidence, and has been demonstrated visually in a particularly convincing manner by the work of McClintock (1929) on maize varieties having aberrant chromosome architecture. It is questionable wisdom, however, to close the mind to the possibility that this machinery is only a small portion of the machinery effective in meiotic and mitotic phenomena. *I should like to suggest that there is a fundamental mechanism for bringing complete genoms into gametes at meiosis and into daughter cells at mitosis. This mechanism, I assume, is a more generalized and less obvious scheme than that usually visualized. Gene-by-gene pairing is thus considered to be a part of a complicated process, occurring wherever possible.*

There is no large quantity of data to support this point of view; but there is evidence, I think, that should prevent our taking the position that the accepted theories of meiosis and mitosis are complete and satisfactory explanations of these phenomena. As examples of what I mean, let me cite first some observations of Sax (unpublished) on the division of the generative nucleus in *Lilium,* where the length of the nucleus is approximately twenty times the width of the tube. Division of each of the twelve chromosomes occurs *without* the formation of an equatorial plate. The daughter chro-

mosomes afterward move considerable distances and are gathered together into the daughter nuclei. One may conclude, therefore, that the equatorial plate, and possibly the spindle fibers, are refinements rather than essential parts of the process of division. Again, I have found in certain *Nicotiana* hybrids where conjugation *does not* occur that the frequency with which viable gametes are formed is higher than that expected by random division into two groups. An example is *N. paniculata* ($N = 12$) $\times$ *N. alata* ($N = 9$), where about ten per cent of the male gametes are functional. The same thing is apparently true of certain other *Nicotiana* hybrids, though in these latter cases I have been unable to show that the apparently good pollen grains are functional. Since, in hybrids between plants where the somatic chromosome number is 24, there is the possibility of 1,352,078 combinations of two sets of 12, while only 4096 of these should be 2 complete genoms, it seems that ordinary segregation is being approximated. Finally, it is not certain that secondary association (see Lawrence, 1931) is prometaphase as distinguished from zygotene, or that it is not concerned in segregation. It seems probable that cases may be found where secondary pairing (i.e., not gene-by-gene pairing) occurs because of general similarity and that these cases are not necessarily to be interpreted as examples of segmental interchange or of polyploidy. Longley (1924, 1926) in *Rubus* and *Citrus*, Chiarugi (cited by Tischler, 1929) in *Artemesia*, and Yarnell (1929) in *Fragaria* may have discovered such instances. Darlington and Moffett (1930) say that such examples (or that of Yarnell, to be exact) of exceptional pairing are probably analogous to that found by Nishiyama (1929) in a triploid *Avena* hybrid, and related by him to segmental interchange. Their interpretation may be correct, but at present it is purely assumption.

At all events, I wish to report some data here that are difficult to fit into the hypothesis that gene-by-gene pairing is the sole tenable hypothesis of chromosome association.

The distribution of the chromosomes of triploids at reduction

It would be expected that in the distribution of chromosomes to the daughter cells at the reduction division of a triploid there should be the ordinary separation of the chromosomes of two genoms plus random segregation of the chromosomes of the odd genom. Such segregation should occur whether there is the *Drosera*-type conjugation or the formation of N trivalents. We find this type of segregation indicated in the counts made on *Canna* (Belling, 1921), on *Datura* (Belling and Blakeslee, 1922), on *Hyacinthus* (Belling, 1924), on *Lycopersicum* (M. M. Lesley, 1926), and on *Petunia* (Dermen, 1930). One also gets the same idea from various counts on species hybrids of the *Drosera* type and from Nishiyama's work (1929) on lagging chromosomes in *Avena* hybrids.

The chromosomes of the functioning gametes of triploids

When the records of the studies of the progenies of triploids are listed, they prove to be a variable lot.

Belling and Blakeslee (1922) found that the functioning egg cells of a triploid *Datura,* from counts on 67 plants resulting from the pollination of the triploid by the diploid, were 12, 13, and 14. in the frequencies 24, 33, and 10. Thus, in spite of the fact that the distribution of chromosome numbers in the pollen (and presumably, therefore, in the egg cells) was binomial, only up to $2N + 2$ plants resulted.

J. W. Lesley (1928) found that in a triploid tomato $(3N = 36)$ pollinated by a diploid, only $2N$, $2N + 1$, $2N + 2$, and $2N + 3$ plants were obtained—the frequencies being 10, 39, 15, and 2, if one distributes the counts on 5 plants which were not classed with certainty as either $2N + 1$ or $2N + 2$. Nine of the twelve trisomics possible were found in approximately equal numbers. The reciprocal cross yielded only 2 plants, both of which were diploids.

Navashin (1929) obtained $2N$, $3N$, and even $4N$ plants from a triploid *Crepis capillaris* selfed, though the aneuploid numbers were comparatively rare.

McClintock (1929), working with a triploid plant of maize, obtained from the cross $3N \times 2N$ a nearly unbroken series from $2N + 1$ to $2N + 7$ in the frequencies 1, 3, 1, 3, 1, 0, 1; while she obtained from the reciprocal cross $2N$, $2N + 1$, and $2N + 2$ plants in the frequencies 27, 6, 2.

Yarnell (1931) found that, in *Fragaria,* triploids $(3N = 21)$ and near-triploids function better as females than as males. From a $2N \times 3N$ mating he obtained 9 fourteen-chromosome, 1 fifteen-chromosome, and 1 twenty-chromosome plants; and from a $2N \times 3N + 1$ mating he obtained no viable plants. But from a $3N \times 2N$ mating he obtained 9 fourteen-chromosome, 11 fifteen-chromosome, 2 sixteen-chromosome, 1 seventeen-chromosome, 1 twenty-chromosome, and 1 twenty-two-chromosome plants; from a $3N + 1 \times 2N$ mating he obtained 5 fourteen-chromosome, 12 fifteen-chromosome, 2 nineteen-chromosome, 2 twenty-chromosome, and 3 twenty-one-chromosome plants; and from a $3N - 1 \times 2N$ mating he obtained 7 fourteen-chromosome, 16 fifteen-chromosome, 4 sixteen-chromosome, and 1 seventeen-chromosome plants.

In contrast to these results, Van Overeem (1921) observed that when triploid forms of *Oenothera* were crossed with diploid forms, the functioning egg cells ranged from 7 chromosomes to 14 chromosomes with no exceptional frequencies. (The reciprocal cross gave only diploid and triploid plants.) De Vries and Bœdijn (1924), from the cross *Oenothera semigigas* × *O. velutina,* obtained a series of progeny having from 14 to 20 chromosomes in frequencies 3, 35, 19, 13, 3, 4, 4. Finally, Moffett (1931) reports observations on 31 seedlings from $3N \times 2N$ apple crosses $(N = 17)$, where the

chromosome numbers ranged without a break from 37 chromosomes to 47 chromosomes inclusive, with frequencies 1, 4, 1, 5, 10, 2, 4, 1, 1, 1, 1.

Again, in contrast to the usual state of affairs, Dermen (1931) obtained no progeny when he made the mating $3N \times 2N$ in *Petunia* ($N = 7$), though he obtained thirty-nine $2N$ plants and twelve $2N + 1$ plants from the reciprocal mating.

The triploid Tabacum of Goodspeed

Goodspeed (1930) has studied the chromosome complement of a plant of *Nicotiana Tabacum purpurea* found among the progeny of a selfed diploid where the flower which produced the seed had been treated with X-rays. At IM the number of chromosomal units varied from 36 to 42. The typical configuration was taken to be 36; and Goodspeed interpreted this as an arrangement comprising $12_{III} + 12_{II} + 12_{I}$.

This interpretation is certainly the logical one, if one takes into consideration all that is known and conjectured regarding the genetic constitution of *N. Tabacum*. But it is always possible that a seemingly logical conclusion is not in accordance with the facts if it must be drawn largely from circumstantial evidence. And in *N. Tabacum* it is difficult to obtain direct evidence on the question as to whether a given chromosomal mass is a trivalent, a bivalent, or a univalent. The 24 chromosomes in the genom of *N. Tabacum* are of different sizes. If one measures each individual body in Ruttle's plate (1928) showing a polar view of mitosis ($\times 4500$) in a haploid, one finds the following lengths, in millimeters (the number of each in parenthesis): 22(3), 21(2), 19(3), 17(5), 16(1), 15(1), 14(4), 13(2), 12(2), and 11(1). Since the chromosomes contract to about one-third of their mitotic length at IM, oval masses are left of such different bulks that mere configuration does not tell whether any one mass is made up of one or of more than one chromosome, for association is usually very compact. In side view at IM and IA it is possible to differentiate in some cases; but these cases always form a minority of the total number. Good diakinesis figures give a somewhat better means of discrimination; but they are difficult to obtain. Thus it seems fair to say that Goodspeed's observations, though probable, are based more upon indirect evidence than upon direct evidence.

EXPERIMENTAL MATERIAL

The H-1 triploid of N. Tabacum

In one of the cultures of *N. Tabacum* grown at the Bussey Institution a triploid plant appeared. As in Goodspeed's case, the variety was *purpurea* As these two cases of triploidy are the only ones thus far discovered in *N. Tabacum,* they awaken the suspicion that perhaps *purpurea* has a tendency in this direction.

The meiotic divisions of this plant and of its progeny were studied in acetocarmine and gentian violet smears, and in paraffin sections from material fixed in Allen-Bouin's solution and in Navashin's solution. The paraffin sections were stained with Heidenhain's haematoxylin.

As in Goodspeed's case, the number of chromosomal bodies at IM varied from 36 to 42. The plates containing more than 36 bodies, however, were relatively rare. About two-thirds of the total number contained 36 masses. No satisfactory material was found at stages earlier than diakinesis; and
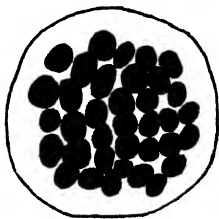
Fig. 1.   Triploid H-1, 1M of PMC showing 36 chromosomal aggregates.   ($\times$ 1800.)

even at diakinesis the figures were not easy to make out. One would be justified, I think, in saying that most of the chromosomal masses appeared to be bivalents, with perhaps here and there an occasional univalent or a trivalent. The frequency with which each type appeared could not be made out. It is not even certain that there were three types.

The association of chromosomes at IM was very close, so that it was impossible to tell whether any particular mass was made up of one, two, or three chromosomes. Size was not a good criterion, as already noted. Careful study of IA, however, gave one the impression that both univalents and trivalents might sometimes be present, as well as bivalents. On the other hand, it is possible to interpret the appearance of the early anaphase stages as being due to variable closeness of association of such a nature that complete separation often occurs earlier than is usual with a normal diploid plant. There were frequent figures wherein the characteristic 8-shaped division was seen as in an ordinary bivalent; there were frequent figures of separate chromosomes; but there were rarely any figures that gave the appearance of chains of three.

Triploid crossed with diploid

The triploid exhibited some self-fertility. After selfing, the capsules contained, on the average, about 20 per cent of the normal complement of seed. A population was raised from seed obtained in this way. The population (31 plants) which was studied most carefully, however, was raised from a mating of the triploid as female with a normal diploid as male. It was thought that, since the male would invariably furnish a complete haploid set of 24 chromosomes, it might be possible in this manner to obtain some

knowledge of the behavior of the functional gametes furnished by the triploid.

Material was obtained from 30 plants. Smears were used to find the variability of the first metaphases. Sectioned material and smears at first anaphase or second metaphase gave us the somatic count. These counts were checked so many times that there is a reasonable degree of certainty for them, with the exception of six cases in which only one count could be made. In these instances the count could not be less than that entered in the record.

Table I gives the frequency distribution of the number of chromosomes of the functioning female gamete. It was made by subtracting 24 from the count of the total number of chromosomes. The number of the plant is given and, in parenthesis, a rough measure of the plant's fertility, made by taking the percentage of seeds in a series of capsules from selfed flowers where 100 per cent is the full complement.

TABLE I.   *Triploid × diploid.   Number of chromosomes in the functioning female gamete and fertility of the plant*

Chromo- some no.	24	25	26	27	28	29	30	31	32	33	34	35	36
Plant no. Fertility in ()		10(15) 11(0)	4(5)		2(0) 17(10) 20(0)	27(15) 29(60)	1(0) 3(40) 9(10) 14(15) 19(40) 21(5) 22(5) 22(20)	26(5)	7(0) 12(10) 23(5) 28(15)	5(tr.) 6(15) 8(5) 16(50) 24(5)	13(5) 15(25) 31(5)	18(0)	

Perhaps the most significant fact concerning the chromosome distribution is the absence of functioning female gametes containing 24 and 36 chromosomes.

The most noteworthy facts in the fertility distribution are: (1) the
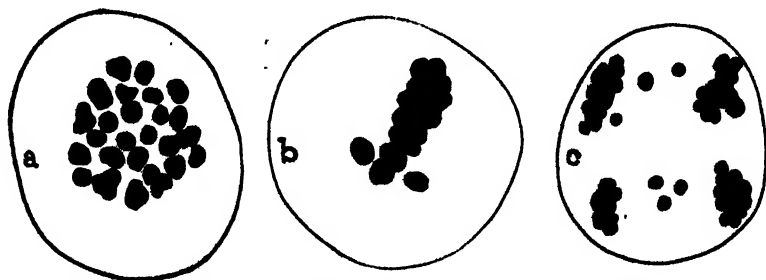
Fig. 2. Triploid × diploid (× 1800), plant 2 (2N + 2). *a,* IM of PMC showing 26 chromosomal aggregates; *b,* characteristic side view; *c,* IIA showing common type irregularity.

distribution of zero fertility over almost the whole range; (2) the two zeros at each end of the distribution where the female gametes were $N + 2$ and $N + 11$, respectively; and (3) the variability in fertility characteristic of plants having the same chromosome number, which indicates that certain

extra chromosomes fit more satisfactorily into the general constitution than do others.

2N + 2 plants. There were two plants in which the female gamete had $N + 2$ chromosomes. Obviously the extra chromosomes were different in the two cases, for plant 10 showed 15 per cent fertility, while plant 11 was wholly infertile.

In plant 10, 26 chromosomal bodies were invariably found at IM, showing that the 2 univalents never paired. The chromosomes formed a flat plate at IM. In a few cases the 2 univalents preceded other chromosomes to the poles. Frequently there were 3 to 6 pairs lagging at IA. In two good figures 3 chromosomes lagged in each plate of IIA. Division did not appear to be by chance. In six plates where the two IIM groups could be counted, five of them were 24 + 26, while only one was 25 + 25. In one very late IIA plate the chromosome complement was 24 + 24 + 26 + 26. Thus there is less fertility shown than should be expected, theoretically, even on the supposition that only 24-chromosome gametes function. It is possible, of course, that this observation may be explained by supposing that one or both of the extra chromosomes have been substituted for chromosomes that belong normally in the genom, and therefore have produced an unbalanced condition in the gamete. But this seems an improbable hypothesis. It is far more likely that the high frequency of 24 + 26 divisions is due to a tendency toward production of normal gametes having the full set of haploid chromosomes. If this be the case, we are driven to the conclusion that the mere presence of the extra chromosomes in the pollen mother is sufficient to produce a lack of balance that persists after the intruders have been eliminated.
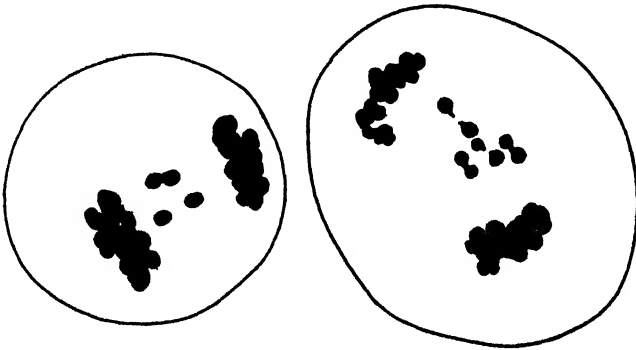
Fig. 3. Triploid × diploid (× 1800), plant 4 (2N + 3). Two views of IA of PMC.

This argument also holds good for plant 11, except that I was able to find only one IIM figure where both plates could be counted satisfactorily. This figure showed 24 + 26 chromosomes. It is also to be noted that while a few IM figures showed 26 bodies, the majority showed 25 bodies, thus leading one to conclude that the 2 extra chromosomes had paired.

2N + 3 plants. Only one plant was found where the functioning female gamete was $N + 3$. It exhibited self-fertility based on a seed complement of 5 per cent. The IM plates all had 27 chromosome groups. A late IA plate showed 24 + 27 chromosomes. A IIM figure showed the same distribution.

2N + 4 plants. There were three $2N + 4$ plants. Plant 2 was wholly infertile. The somatic count was based upon only 1 satisfactory figure of the two plates at IIM. These showed 26 + 26 chromosomes. The most frequent count at IM was 26, though plates with 27 were fairly common, and plates with 28 were not rare. These observations can be interpreted as arising from the production of 2, 3, or 4 univalents and 2, 1, or 0 trivalents; but there is considerable evidence that the four extra chromosomes formed two pairs in the majority of cases, with one pair plus two univalents or four
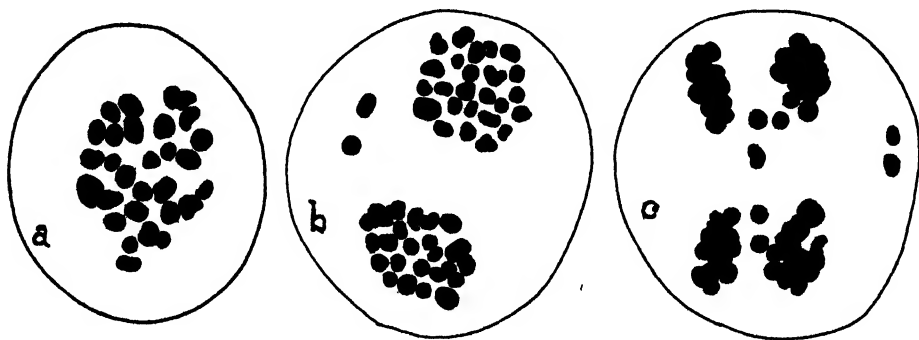
Fig. 4. Triploid × diploid (× 1800), plants 2 and 17 ($2N + 4$). *a*, IM of PMC of plant 2; *b*, IIM of PMC of plant 17, 25 + 2 + 25 chromosomes; *c*, IIA of PMC of same plant.

univalents as less likely occurrences. This evidence comes from the early first anaphases. The IM plates are narrow, indicating closeness and regularity in pairing. The early IA's usually show 2 lagging pairs, though occasionally 2 or 4 chromosomes are seen in dichotomous division preceding the other chromosomes to the poles. In one instance eight chromosomes were seen lagging.

Plant 20 was also wholly infertile and behaved like plant 2, though exhibiting less variability. IM always showed 26 chromosomal bodies. The somatic count was again based on a single pair of IIM plates; but these plates were very satisfactory and showed unmistakably a 26 + 26 division.

Plant 17, on the other hand, was given a 10 per cent fertility rating. Some dozen or so IM plates showed the single figure of 26 chromosomal bodies. One pair of IIM plates showed a 26 + 26 count. But this was not the characteristic behavior of plant 17. In several good pairs of IIM plates, 25 chromosomes were seen at each pole, while 2 were left in the center. In all second division plates examined, this pair of chromosomes was invariably found out in the cytoplasm and did not figure in the division.

2N + 5 plants. The two 2N + 5 plants, numbers 27 and 29, were estimated to have the fertility percentages 15 and 60, respectively—the latter being the most fertile plant found in this population.

In plant 27, 26 bodies were usually found at IM. In rare cases there were 27. It was characteristic of the IA in this plant that one pair of chromosomes was found to be lagging. Occasionally this pair was not included in the daughter nuclei. IIM counts, where only one plate could be counted, were nearly always 26 + 1 standing alone. Plates where both figures could be counted were 27 + 26, 25 + 28, 27 + 1 + 26, 27 + 1 + 26, 25 + 2 + 26.

In the highly fertile plant, plant 29, 8 satisfactory IM figures showed 6 counts of 26 and 2 of 27. One good IA showed 53 chromosomes. IIM counts were 26 + 27, 26 + 27, 25 + 27(?), 25 + 2 + 26. It was not characteristic of this plant to show lagging chromosomes at IA, though this was sometimes seen. But there were lagging chromosomes in nearly all the IIA plates. Usually there were 2 laggards in each figure, making 4 in all; but as many as 6 were seen on one side, as against 4 on the other side.

Thus, these two plants did not show the same type of disturbance. In one the irregularity was found at the first division; in the other it was found at the second division. There must have been at least one trivalent at IM in both instances. It is possible that all the five extra chromosomes appeared as trivalents and univalents; but I gained a decided impression that four of them were usually paired.

2N + 6 plants. The 2N + 6 group included eight plants. In this fact there is perhaps a suggestion that six, rather than twelve, is the basic chromosome number for *Nicotiana*.

The lowest fertility (0) was that of plant 1; yet there was nothing in the meiotic division to indicate a high degree of irregularity. IM usually showed 28 bodies, though one was found with but 27. Several side views at IM showed 2 chromosomes preceding the others to the poles. If this did not occur, 2 chromosomes were found lagging. Two good IIM figures were each 26 + 2 + 26.

Two plants, numbers 21 and 22, showed 5 per cent fertility. In ten IM plates of plant 21, there were the following counts: one of 26, one of 27, and eight of 28 chromosomal bodies. One pair was almost always thrown out entirely at the first division. IIM plates were nearly always 26 + 2 + 26, though occasionally plates of 27 + 2 +25 were found. In numerous IM counts of plant 22, about half showed 29 and half 30 chromosomal bodies. Generally there were 6 laggards at IA. Only 2 satisfactory IIM plates were found. They were 24 + 6 singles + 24.

Plant 9, showing 10 per cent fertility, was the most irregular of this lot. It is not absolutely certain that the somatic count is correct, for one IIM from a gentian violet-iodine smear showed a count of 26 + 3 scattered + 24 + 2 scattered; and a second showed 28 + 27. I have interpreted these

counts as due to premature division of one chromosome, however, as the other counts did not agree. There were other counts of 27 + 27 (several), 26 + 28, and 24 + 26 with 4 scattered. Moreover 6 chromosomes, apparently univalents, were often seen as laggards at IA.

Two plants, numbers 14 and 25, showed 15 and 20 per cent fertility, respectively. IM plates usually showed 27 bodies. There were a few with 28 and 29, but none with 30 bodies. Two chromosomes were practically always thrown out at the first division. One late IA gave the count 26 + 2 + 26. There were a number of IIM figures where only one plate could be counted. They were 26 grouped + 2 singles. Plant 25 showed either 28 or 29 chromosomal bodies at IM. The best IIM plates were 27 + 1 + 26,
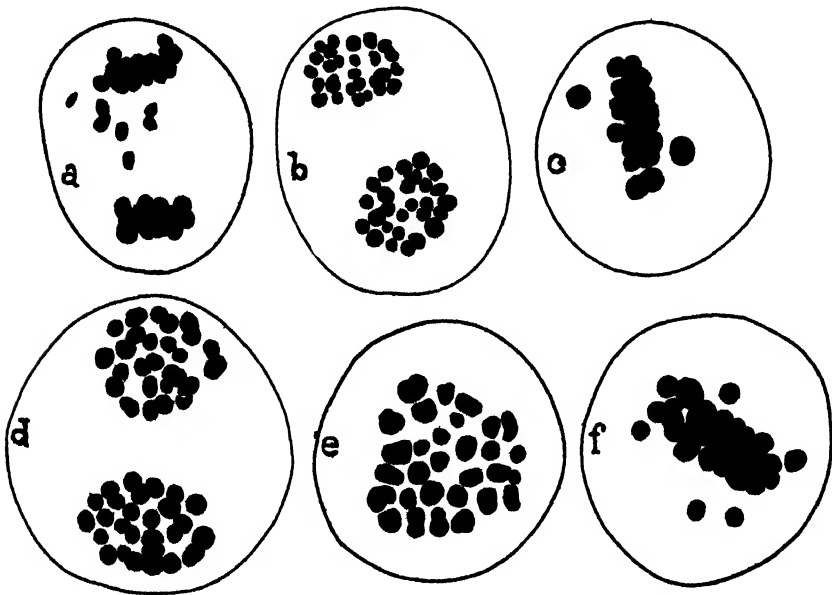
Fig. 5. Triploid × diploid (× 1800), plants 14, 19, and 22 (2N + 6). a, IA of PMC of plant 14; b, IIM of PMC of plant 14 showing 27 + 27 chromosomes; c, same plant side view IM; d, IIM of PMC of plant 19, 27 + 27 chromosomes; e, IM of PMC of plant 22 showing 30 chromosomal aggregates; f, side view of IM of same plant.

27 + 27, and 28 + 1 + 25; but from side views of IA plates one was impressed with the fact that only one chromosome behaved as a univalent. The IIA was very irregular. Laggards on each side in a random count of eight good plates were: 3–1, 3–3, 3–2, 1–1, 4–3, 1–0, 2–5, and 3–3.

The last two plants of this group, numbers 3 and 19, showed 40 per cent fertility. Plant 3 nearly always showed 30 chromosomal bodies (rarely 28 or 29) at IM. Good IIM figures were lacking in this material. The somatic count was based on one good figure. It was 24 + 6 + 24. Plant 19, on the other hand, showed only one count of 30 at IM. The most frequent count, by far, was 27 (80 per cent). Chromosomes were rarely thrown out at the

first division, though one count at IIM gave the figures 26 + 2 + 26. Other counts at this stage were 28 + 26, 27 + 27, and 28 + 22. Several single figures at IIM showed 27 on the side that could be counted.

$2N + 7$ *plants.* Only one plant, number 26, had $2N + 7$ chromosomes (fertility 10 per cent). In ten IM plates there were five with 27, two with 28, two with 29, and one with 30 chromosomal bodies. In just over half of the IM plates, from 1 to 4 chromosomes, apparently univalents, preceded the others to the poles. Division appeared to be remarkably regular, laggards being seen only occasionally in side view. Yet the satisfactory IIM plates used for the somatic count showed from 1 to 3 chromosomes outside the two groups—viz., 28 + 1 + 26, 27 + 1 + 27, 27 + 28, 26 + 3 + 26, 27 + 2 + 26.

$2N + 8$ *plants.* There were four plants in this category, numbers 7, 12, 23, and 28. Their fertility ratings were 0, 10, 5, and 15, respectively. In plant 7 the counts at IM were nearly always 28, with occasionally a 27 or 26. No count of over 28 was obtained. The first division was regular, except that one chromosome often went to each pole before the others. Counts at IIM were 27 + 2 + 27, 30 + 26, 28 + 28, 28 + 28, 30 + 26.

In plant 12 the first division was also very regular, except that one chromosome was often seen preceding the others to the pole; but here there was also a second pair (behaving like univalents) usually seen as laggards. At IM the usual count was 28 bodies; more rarely it was 27 or 29. The count at IIM was 27 + 2 + ?, 28 + 2 + 26, 27 + 2 + 28(?), 27 + 2 + 27.

Plant 23 exhibited wide variations in the counts at IM, ranging from 27 to 32. In 25 counts these classes appeared in frequencies 1, 12, 6, 1, 2, 2. It might be supposed that in the two instances where 32 bodies were counted at IM, there were 8 univalents. But this did not appear to be the case. In both these plates there was the clearest evidence that I have seen of the presence of 4 trivalents and of 2 early divisions. At IA there were usually 2, 4, or 6 chromosomes lagging when seen in side view. It is possible that this plant is $2N + 7$, for two apparently satisfactory IIM plates gave counts of 27 + 28. It is listed as $2N + 8$, however, since most of such figures were 28 + 29, 26 + 4 + 26, and 26 + 2 + 28, 27 + 1 + 28. One rather irregular count was 26 + 7 + 23. IIA was very irregular, with 1 to 4 lagging chromosomes on each plate.

Plant 28 nearly always yielded IM plates with 28 bodies, counts of 27 and 29 being found in rare cases. But for one count of 28 + 28 at IIM, the counts (5 were satisfactory) were 27 + 2 + 27. The 2 chromosomes outside of the IIM groups were in the periphery of the cell.

$2N + 9$ *plants.* Plant 5, showing only a trace of fertility, exhibited considerable variability in its IM groups. Ten counts, taken at random, show 28, 29, and 30 bodies in the frequencies 2, 6, and 2. At IA, 2 chromosomes generally are to be found preceding the others to the poles. Frequently 5 lagging chromosomes are found at IA. But few satisfactory counts were

made at this stage. The best two were $28 + 5 + 24$ and $30 + 27$. There were 9 laggards in one plate.

In plant 6, showing 15 per cent fertility, there was the same type of irregularity that was found in plant 5. Out of 10 plates of IM there were 7 showing 28 bodies; in addition, there were 2 showing 30 and 1 showing 29. In the IM plates 2 chromosomes were regularly seen preceding the others to one pole, while from 1 to 3 chromosomes were seen at the other pole. In both these plants there was great irregularity at IIA, since from 2 to 5
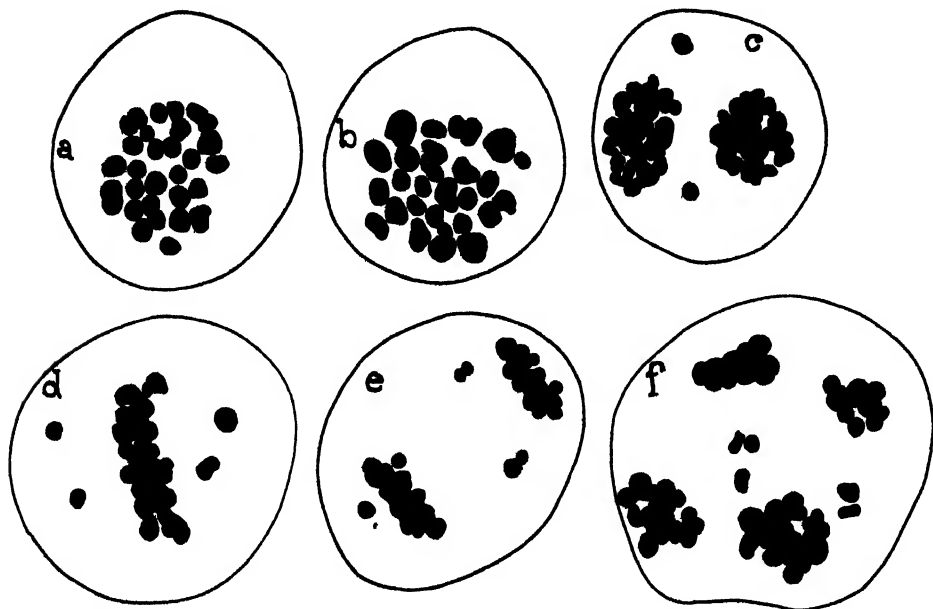
Fig. 6. Triploid $\times$ diploid ($\times$ 1800), plants 6, 8, and 16 ($2N + 9$). *a*, IM of PMC of plant 6 showing 30 chromosomal aggregates; *b*, IM of PMC of same plant showing 28 chromosomal aggregates; *c*, IT of PMC of same plant showing a common elimination of 2 chromosomes; *d*, side view IM of PMC of plant 8 showing early separation of 4 chromosomes; *e*, IT of same plant; *f*, IIT of PMC of plant 16.

chromosomes were laggards on each spindle. The only good IIM figures were $28 + 29$, seen twice; but several single IIM plates showed counts from 27 to 30.

Plant 8, showing 5 per cent fertility, was the only plant of this population that did not show a sharply modal arrangement at IM. Here counts of 28, 29, 30, and 31 were found with equal frequency. It was characteristic of this plant that from 2 to 5 chromosomes were not arranged on the plate. Instead, they were invariably found above or below the main group. The usual arrangement above and below was 1 and 1, or 1 and 2; less frequently the arrangement was 2 and 2, or 2 and 3. The usual IIM counts were $28 + 29$ or $30 + 27$. IIA was irregular, from 1 to 3 laggards being found on each plate.

Plant 24, also showing 5 per cent fertility, was not studied so carefully as the others because only 2 slides were satisfactory. IM usually showed 29 bodies, though 30 were sometimes found. Only one good IIM figure was found. It was 28 + 29.

Plant 16, on the other hand, was studied very carefully, as it showed 50 per cent fertility. It was the only plant possessing more than 54 chromosomes that showed over 15 per cent fertility with the one exception of plant 15, having 58 chromosomes and exhibiting 25 per cent fertility. A random sample of twenty-five IM counts ranged from 27 to 30, with the frequencies 7, 14, 2, 2. Divisions indicated by IIM figures were 28 + 1 + 27, 28 + 1 + 28, 27 + 1 + 29, 28 + 1 + 28, 30 + 27.

Two satisfactory diakinesis plates showed 28 and 29 groups, respectively. Only bivalents were found. In the 28-group figure there should be at least one trivalent, and in the 29-group figure there should be at least one univalent; but if such were present, they must have been at the periphery, where distinctions are difficult to make.

2N + 10 plants. Plants 13 and 31 of the three of this group were recorded as having 5 per cent fertility. In plant 13 ten counts, taken at random
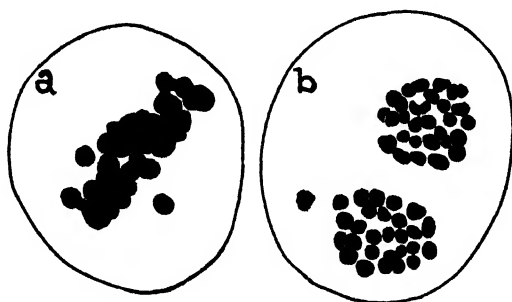
Fig. 7. Triploid × diploid (× 1800), plants 13 and 31 (2N + 10). *a,* side view IM of plant 13; *b,* IIM of PMC of plant 31 showing 28 + 30 chromosomes.

at IM, show the number of bodies to range from 27 to 33, with frequencies 1, 1, 5, 1, 0, 1, 1. Thus, while the mode is at 29 and indicates pairing, in rare cases 10 univalents may be present. One satisfactory diakinesis figure showed 29 groups that were apparently all pairs. The characteristic appearance of IM side views was a pair of singles on each side of the plate, the configuration seen so often in these plants. At IA three pairs were often seen lagging at the equatorial plate, in addition to one or more singles. Only one trustworthy figure of IIM was counted. It was 27 + 3 + 28.

Plant 31 also had a modal condition of 29 bodies at IM, with an occasional 28 or 30. Two good IIM plates showed counts of 29 + 29 and 28 + 30; and an excellent late IIA showed a count of 29 + 29 + 29 + 29.

Plant 15, which was 25 per cent fertile, had a sharp modal condition of 28 bodies at IM, with a few plates counting 27, 29, and 30. The common

condition at IA was one with from 2 to 5 singles and 1 or 2 pairs lagging. The ordinary count at IIM was $28 + 1 + 29$ or $27 + 1 + 30$. At IIA from 1 to 7 chromosomes were seen as laggards at each plate.

2N + 11 plants. The single plant with $2N + 11$ chromosomes was completely sterile. IM counts showed a mode at 31 bodies; but counts of 28 and 29 were not rare, thus indicating some trivalency. At IA, 1 or 2 pairs and from 1 to 4 singles were commonly seen as laggards; but in no case did these laggards show a total count of 11. Counts at IIM were $27 + 32$, $30 + 29$, $29 + 30$.

Triploid selfed

Eighteen plants were raised from the triploid selfed. Nine of them were completely sterile, two of the group producing no pollen mother cells. Thus the fertility of the selfed population was much lower than that of the triploid × diploid population. Of the other nine plants, seven showed about 5 per cent fertility, one showed 20 per cent fertility, and one showed 75 per cent fertility. Smears were made of eleven of these plants and counts recorded of 1M. Six of the plants showed counts of 28 bodies with little variation. Ten counts per plant showed 2 aberrant groups of 29 on one plant and 3 aberrant groups of 30 on another plant. The remainder were 28. In addition, one plant showed only groups of 20, one showed only groups of 27, one showed groups of 29 (with an occasional 28), one showed groups of 30 (with an occasional 29), while one showed a variation from 30 to 33. A good deal of irregularity was seen in the second division, but there was a noteworthy regularity in the first division. The plant showing 26 groups at IM apparently had only bivalents. No univalents were seen at IA, and the division was $26 + 26$. The plant showing 27 groups also appeared to have only bivalents at IM. The divisions were $27 + 27$, $26 + 2 + 26$, and $26 + 28$. In the plant showing 29 groups at IM, there was one obvious univalent. The single satisfactory IIM found was $29 + 28$. The plant having usually 30 groups at IM appeared to have 3 univalents. The single good IIM found was $29 + 30$.

DISCUSSION AND SUMMARY

The functional female gametes of the *N. Tabacum* triploid H-1, as shown by the study of the triploid × diploid population of 30 plants, had a chromosome distribution that is not wholly removed from what one should expect on the basis of pure chance. The mode is 30 chromosomes. There are 8 plants under the mode and 14 plants over the mode. The range is from 26 chromosomes to 35 chromosomes without a break. And no plants appeared with $2N$ or $3N$ chromosomes, as has been the case with so many hybrids between closely related species where the parents contributed N and $2N$ chromosomes, respectively. Yet there are peculiarities in the distribution which appear to indicate that selective factors have been at work. There

is a secondary mode at 33 chromosomes (female gametes) and a marked
deficiency of 29-chromosome and 31-chromosome gametes. Perhaps there
are no more functioning 30-chromosome gametes than one should expect,
but there is a slight indication that this is the case. The idea that there is a
chance distribution, however, is strengthened by the great differences in fer-
tility exhibited by plants having the same chromosome number. It is common
knowledge that fertility is easily affected by genetic disharmony; therefore
it appears probable that extra chromosomes of a gamete having a given
number are not usually the same combinations.

Though there was no trend toward the production of N-chromosome
gametes in the original triploid, there was such a tendency in at least one,
and perhaps both, of the $2N + 2$ plants. It is suggested that the low fer-
tility of such gametes is due to the persistence of a lack of balance induced
by the presence of extra chromosomes in the immature germ cells.

The association of the chromosomes in the triploid was usually such that
36 chromosomal masses could be counted at IM. The count occasionally ran
above this number but never was greater than 42. The plates were flat, and
division appeared to be regular. Some of the masses could be interpreted
as trivalents, but this was never certain, as I have already noted in the text.
Univalents were occasionally seen at IA, but no trivalents. The behavior at
IA was that to be expected of 36 bivalents.

The entire group of data on the progeny of the triploid, particularly those
from the triploid crossed with the diploid, appeared to strengthen this hetero-
dox conclusion. I do not mean to say that univalents and trivalents were
not indicated. They were. Univalents, behaving as isolated entities, were
observed frequently. A few undoubted trivalents were noted at IA (about
25 in a complete examination of over 500 slides). Moreover some of the
numbers of the chromosome masses seen at IM are such as to require a
limited number of univalents and trivalents. But the picture gained from
the study of thousands of figures was that of secondary pairing occurring
with great frequency.

The argument runs somewhat as follows. The count at IM nearly
always showed a sharp modal condition at a figure more simply interpreted
by secondary pairing of most of the extra chromosomes than by 24 primary
pairs plus trivalency and univalency. The variation from the modal condi-
tion practically always showed a rise in the IM counts; and plants which
showed this type of behavior always showed a variable lot of unmistakable
univalents.

The first meiotic division was usually fairly regular; it was the second
division that usually showed the higher degree of irregularity. The indica-
tion was a division of bivalents accompanied by the separation of a
smaller number of univalents than the number of extra chromosomes
present. The univalents behaved as univalents are expected to behave—as
isolated bodies. At the anaphases the univalents often were seen as laggards,

though one or two pairs (characteristically one in certain plants) often preceded the other chromosomes to the poles. But in addition to the univalents, bivalents were seen in nearly every anaphase figure that had any laggards whatever, while trivalents were very rare. And finally, the tendency toward a dichotomous division appeared to be far greater than that expected by chance, without prejudice as to whether or not the resulting gametes could function.

There is also a minor point to be noted here. Plants with the same chromosome number indicated by IM counts and IA behavior that some chromosomes pair more easily than others.

This conclusion is very unorthodox. N trivalents have often been found in triploids; and where the count is N-x, there are usually x univalents. Theories of meiosis based on genetic tenets would seem to require the formation either of trivalents or univalents in a triploid. Only a few cases of apparent conjugation of non-homologous chromosomes have been reported, and these have been questioned, or have been interpreted (as in the Pomoideae) by assuming polyploidy. But polyploidy from a basic number less than 12 does not fit the case very satisfactorily in *N. Tabacum*. In *Nicotiana* there are species with 9 and with 10 chromosomes, but from the size relations of the chromosomes there seems to be more justification in assuming chromosome fusion on a 12 basis than in assuming a 6 or a 9 basis. There is some slight evidence of a 6 basis in the behavior of our triploid and in the behavior of the hybrids between *N. Tabacum* and the 12-chromosome species *N. sylvestris*, *N. Rusbyi* (or, *N. tomentosa*), and *N. glutinosa;* but if this were the case, there should be some internal pairing other than the *Drosera*-type pairing in the above hybrids, and this is not the case. It may be objected that the same argument applies about these hybrids, if the conclusions I have drawn concerning the H-1 triploid are true. This is, of course, the fact. I can only answer that perhaps there is an additional variable concerned when dealing with the above species hybrids that is not present in the triploid.

I do not wish to be dogmatic in the interpretation of the results reported in the present paper. At the same time it appears to me to be unwise, in our current state of knowledge, to become wedded to the conviction that gene-by-gene pairing of chromosomes is all there is to chromosome pairing. Undoubtedly gene-by-gene pairing occurs and is the fundamental mechanism in the distribution of the hereditary units; but perhaps there is another force or mode of action that brings the chromosomes into association through general similarities as a prerequisite to the essential type of pairing which then occurs if possible. As an illustration of what I mean, let me cite some other cytological facts about *N. Tabacum,* the plant with which we are dealing here. The *Tabacum-sylvestris* and the *Tabacum-tomentosa* (or *Rusbyi*) hybrids show *Drosera*-type pairing ($12_{II} + 12_{I}$). Goodspeed and Clausen, Brieger and Kostoff have become more or less firmly convinced from this fact and from the behavior of the hybrids that *N. Tabacum* is a *sylvestris*-

tomentosa tetraploid.   Kostoff even believes, with some reason, that he has partially reconstituted *N. Tabacum* by obtaining a 48-chromosome plant that is partially fertile by crossing it with the hybrid *sylvestris-Rusbyi* (the latter is probably only a variety of *tomentosa*).   But if these conclusions are correct, how is one to interpret the fact that *Drosera*-type pairing, with some variation, is found in the hybrid between *N. Tabacum* and *N. glutinosa*, another nearly related 12-chromosome species?   How is one to interpret the fact that *Drosera*-type pairing is found in the *Tabacum-glauca* hybrid— certainly a very distant union?   How is one to account for the variable amount of pairing found when *N. Tabacum* is crossed with the very distant species *N. alata* and *N. Sanderae?*   And finally, how can one explain the fact that under greenhouse conditions and the increasing light of spring, 24 haploid chromosomes from *N. Tabacum* pair completely with the 24 haploid chromosomes from *N. rustica* in that hybrid?

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CHROMOSOME STUDIES IN *ZEA MAYS* L.

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INTRODUCTION

In *Zea* a considerable number of variations from the normal chromosome number have been observed. The fact that these variations in number occur is of importance to the student of maize genetics in that they may give rise to abnormal ratios that cannot be explained by normal genetic segregation. It is therefore of considerable importance to have both a cytologic and a genetic understanding of the material with which one is working.

Variability of chromosome number is not by any means peculiar to *Zea*. It has been reported in a number of species and seems to be of more or less common occurrence in nearly every species that has been thoroughly studied. It is the purpose of this investigation to study the chromosome number of the maize " testers " which are being used in studies of linkage relations at the Minnesota Agricultural Experiment Station.

Maize has been the subject of much cytological investigation. Kuwada (1915) found the haploid chromosome number to vary from 9 to 12. In later work (1925) he concluded that 10 haploid was the number for *Zea Mays*. Kiesselbach and Peterson (1924) made an extensive study and found no deviation from 10 haploid. Longley (1924), in a cytological study of maize and maize relatives, reported the following chromosome numbers: *Zea Mays*, 10 haploid; *Euchlena perennis*, 20 haploid; *Euchlena mexicana*, 10 haploid; *Coix lachrima-jobi*, 10 haploid; and the *tripsocii*, approximately 35 haploid. Reeves (1925) reports 10 haploid as the chromosome number for several varieties, and as high as 12 for Black Mexican. Fisk (1925, 1927) studied 25 strains of maize and reported a number of 10 haploid and 20 diploid. Longley (1927) reports 10 haploid as the normal number for maize, though he observed extra chromosomes in certain varieties. Randolph (1927) made an extensive study of the chromosome number in maize, using 19 varieties and 24 genetic cultures. He concluded that 10 haploid was typical for maize, but he found extra chromosomes in Black Mexican and Golden Bantam and in seven genetic cultures. In these the numbers ranged from 21 to 28 diploid.

MATERIALS AND METHODS

Cultures used in this study. A large part of the genetic cultures used in this investigation was originally secured from Dr. R. A. Emerson of Cornell in 1923. In some cases it has been necessary to cross certain of the cultures

with earlier maturing strains, in order to shorten the growing season sufficiently to secure mature seed.

Ten linkage groups are now known and data regarding these have been summarized by Emerson, with a list of the genes of each group and their relationships. In table 1 the cultures have been arranged in the order used by Emerson.

TABLE I. *Maize cultures used and chromosome counts*

Lab. no.	Group	Genotype	No. of somatic counts	No. of microsporocyte counts	Diploid number	Haploid number	No. of plants
1	C–WX	*AcR, C tester*	40	42	20	10	4
2	C–WX	*vl*	40	34	20	10	2
3	C–WX	*vl*	28	36	20	10	1
4	R–G₁	*ACr, R tester*	56	56	20	10	5
5	Su–Tu	*Tw Tw Sw*	45	26	20	10	3
6	Su–Tu	*gl₁*	37	6	20	10	4
7	B–LG	*gl₂ fl*	27	20	20	10	2
8	B–LG	*ts₁ × Ts₁ls₁*	32	9	20	10	3
9	B–LG and Y–PL	*Ylg*	37	18	20	10	3
10	P–BR	*ts₂ × Ts₂ts₂*	40	22	20	10	3
11	P–BR	*br*	34	12	20	10	3
12	RA–Gl₁	*Bngl₁*	34	29	20	10	2
13	RA–Gl and C–WX	*wxsl*	46	—	20	—	—
14	PR–V₂	*bm*	40	9	20	10	3
15	PR–V₂ and C–WX	*ACR pr wx, Pr tester*	27	—	20	—	—
16	PR–V₂ and C–WX	*prsh*	56	11	20	10	2
17	A–TS₁	*A tester aCR*	—	15	—	10	4
18	A–TS₁ and B–LG	*A tester aCRlg (Emerson)*	34	102	22	11	6
19	—	*Bantam Evergreen*	48	11	20 and 26	10	2

Cytological technic. The seed used to obtain root-tip material was germinated between wet blotters, and the tips were taken when the primary root had attained a length of about an inch. The tips were killed in Bouin's killing fluid as modified by Allen. They were then imbedded in paraffin and sectioned 15 microns thick. Safranin and gentian violet stains were used.

A part of the microsporocyte material was grown in the greenhouse during the winter of 1929–1930, and the rest was taken from the field in the summer of 1930. All of this material was examined with iron-aceto carmine by Belling's method (1921). This method yielded numerous excellent preparations of the metaphase chromosomes in the microsporocytes, and a considerable part of the study of the material was made in this manner. The technic used was similar to that used by Belling. When a satisfactory preparation was secured, the edges of the cover slip were sealed with shellac. This kept the preparation in good condition for a period of many weeks.

The material selected for imbedding was placed in Bouin's killing solution, as modified by Allen, and later imbedded in paraffin. This material was sectioned 15 microns thick. Staining was done with safranin and gentian violet.

EXPERIMENTAL RESULTS

The cultures are arranged in table 1 according to Emerson's order of the groups and are numbered accordingly. The numbers in the first column are the laboratory serial numbers. In the next column is the linkage group to which the culture belongs, while the third column contains the genotypes of the various cultures. The next two columns show the number of counts made—in the first the number of diploid counts from root tip material, and in the second the number of haploid counts from microsporocyte material. The next two columns show the chromosome number found for the various cultures, the first showing the diploid number and the second the haploid
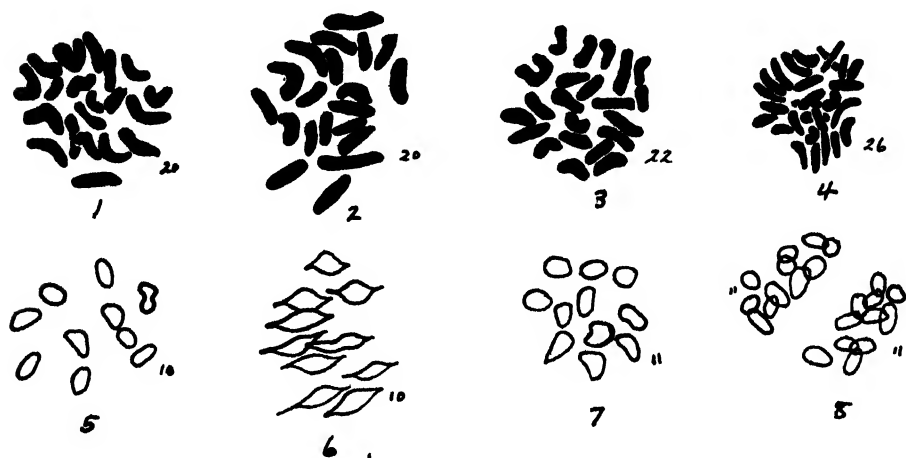
Fig. 1–8. *Zea Mays*. Fig. 1. Metaphase of *wx sl*, culture 13, showing 20 chromosomes. From root-tip preparation. ✕ 2100. Fig. 2. Metaphase of *ts₂ ✕ Ts₂ts₂*, culture 10, showing 20 chromosomes. From root-tip preparation. ✕ 2100. Fig. 3. Metaphase of *A tester* (Emerson), culture 18, showing 22 chromosomes. From root-tip preparation. ✕ 1800. Fig. 4. Metaphase of Bantam Evergreen, culture 19, extra chromosome preparation, showing 26 chromosomes. From root-tip preparation. ✕ 1350. Fig. 5. Metaphase of first division in microsporogenesis, showing 10 chromosomes. This is the *A tester*, culture 17. (Brown.) End view, ✕ 1800. Fig. 6. Metaphase of first division in microsporogenesis, showing 10 chromosomes. This is *vl* (Demerec), culture 2. Side view, ✕ 1800. Fig. 7. Metaphase of *A tester* (Emerson), culture 18, showing 11 chromosomes in microsporogenesis. End view, ✕ 1800. Fig. 8. Anaphase of *A tester* (Emerson), culture 18, showing 11 chromosomes in microsporogenesis. Side view, ✕ 1800..

number. In the last column are listed the number of plants from which microsporocyte material was secured.

From one to six plants of each culture were examined in microsporogenesis, except in two cases. In these no material was obtained. In every culture except the *A tester, aCRlg,* obtained from Emerson, the chromosome number was found to be 10 haploid (fig. 5, 6). In this *A tester* the number was 11 haploid (fig. 7, 8). One hundred and two counts were made and no

variations from eleven were observed. In all, 54 plants were observed in microsporogenesis and 426 counts of the chromosomes made. These observations were made during various meiotic stages, including diakinesis and metaphases and anaphases of both the first and second divisions. No irregularities in chromosome behavior were observed.

Seven hundred and one observations were made in the root tip material, and in all the cultures except Emerson's *A tester* and the Bantam Evergreen variety, the chromosome number was found to be constantly 20 (fig. 1, 2). In this *A tester* there were 22 chromosomes in every count made (fig. 3). In the Bantam Evergreen variety the normal number is 20 diploid. Root tips from eleven seeds were examined and in ten of these the number was found to be regularly twenty. In one, however, there were 26 chromosomes (fig. 4). As this variety originated from a Bantam Evergreen cross, it might be expected to vary somewhat in chromosome number, since both of the parental varieties have been found to vary.

It is of interest to note that, while Emerson's *A tester* had 22 diploid and 11 haploid chromosomes, the *A tester* secured from Brown had the normal haploid number (fig. 5). Emerson's *A tester* is of special interest from another standpoint. In crosses using this *A tester* and another culture with purple aleurone, Hayes and Brewbaker (1926) obtained deviations from the expected 3:1 ratio of purple to red seeds and suggested that these might be explained by linkage between *Pr* and *C* or *R*. As similar deviations have been obtained on unpublished material in segregations where only purple and red aleurone are concerned, they have become convinced that linkage was not the explanation. Wentz (1930) made crosses similar to those of Hayes and Brewbaker and obtained the same results. He suggests the possibility of a genetic factor in addition to *Pr* which was affecting the inheritance of purple and red seeds. Whether or not this is the case had not been proved. It is possible that the extra chromosome in the *A tester* may be responsible for these purple-red ratios, and in further studies this matter will be investigated.

DISCUSSION

The results of this investigation agree well with those of other workers on this subject. Numerous types and varieties of maize have been investigated. In relatively few cases has the chromosome number been observed to vary from the normal, 20 diploid or 10 haploid. The same is true for this investigation. Out of 19 types studied only two were found to vary from the normal. Thus this work may be taken as further evidence that 20 is the normal diploid number for *Zea Mays*.

It has been observed by certain investigators, chiefly Randolph (1927) and McClintock (1929), that the diploid chromosomes vary in length. Such was observed to be the case in this study. The longest were approximately twice the length of the shortest. The lengths of the rest grade between the shortest and longest, there being a pair of each length. While the principal

purpose of this investigation was not a morphological study of the chromosomes, a few points of interest were noted. In certain of the cultures the chromosome lengths varied far less than in others. In some cases the chromosomes were markedly thick, as compared with others in which they were slender. In only one case was a satellite observed. The relative lengths and thicknesses of the chromosomes are markedly constant within cultures.

The *A tester* with 11 pairs of chromosomes is of special interest. All eleven pairs stained in the same manner, and a constant number of 11 was observed in reduction division. This culture has been crossed with others to determine if possible whether factors carried in the extra chromosome may cause deviations in normal ratios.

SUMMARY

1. The typical diploid number of chromosomes in the cultures of maize studied was 20 except in one case.

2. In one case the number was constantly 22 diploid and 11 haploid.

3. One plant with more than the typical number was observed in the Bantam Evergreen variety.

4. No variation of chromosome number was observed in six individuals of the exceptional genetic culture (culture 18, table 1).

5. In the exceptional culture the extra chromosomes could not be distinguished by any morphological peculiarities.

6. In the 26-chromosome plants of the Bantam Evergreen variety there appeared to be a greater number of short chromosomes than in the normal complement.

7. Relative lengths and thicknesses of the chromosomes appear to be quite constant within cultures.

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STUDIES ON THE PRECIPITIN REACTION IN PLANTS. IV. THE QUESTION OF ACQUIRED REACTIONS DUE TO GRAFTING

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In a series of papers Kostoff (1928, 1929, 1930, 1931) claimed to have demonstrated acquired immunity in plants. His assertion was based mainly on the behavior of the " precipitin reaction " in certain grafted solanaceous plants. Reactions occurring when extracts of two species of plants are tested by the ring method were termed " normal precipitin reactions." When extracts of each member of a graft combination were similarly prepared and tested and the resulting precipitation was considered greater than the " normal precipitin reaction," it was termed an " acquired precipitin reaction " and interpreted as indicating a case of acquired immunity in plants.

On this basis other phenomena taking place in vivo were interpreted by Kostoff as antigen-antibody reactions. Several types of such phenomena should be mentioned:

1. The chloroplasts agglutinated in graft unions of *Nicotiana glauca* on *Capsicum pyramidale* on both sides of the callus.

2. Grafted plants of *N. glauca* on *C. pyramidale* grew well until 4–5 weeks after grafting, when the scions died. Repeated attempts to graft new scions of the same species on these old plants resulted in failures. The conclusion reached on the basis of this evidence was: " Since the inhibition of shoots approximately 4–5 weeks after grafting coincides with the period of greatest antibody production, failure of subsequent grafts on those stocks which had already killed their scions is undoubtedly due to the antibodies induced in the stocks by grafting."

3. When *Nicotiana Tabacum* was grafted on *Datura Wrightii*, the scions grew normally until the time of flowering. At this period the flowers did not develop normal corollas; they were characterized by having a greater tendency to wither than those of normal plants.

4. When *Nicotiana Langsdorffii* was grafted on *Solanum nigrum*, there were noticeable disturbances of the meiotic divisions; abnormal reduction and equational divisions of the pollen mother cells resulted in the abortion of about 50 per cent of the pollen grains as compared with control plants.

Kostoff's method of demonstrating acquired immunity was a radical de-

parture from previous methods used in attacking this problem. A number of grafts were made among species of the Solanaceae; 4–6 weeks after grafting, the plants were tested, and if the " normal precipitin reaction " had risen beyond that of ungrafted plants of the same species, the stock and scion were assumed to have " acquired precipitins." The observed rise of the " precipitin reaction " was the essential feature of Kostoff's work and served as a foundation for its interpretation as an antigen-antibody reaction.

The problems arising from such an interpretation were so fundamental and so important that an investigation of the chemical nature of the " normal precipitin reaction " in plants and a repetition and extension of the work on the effect of grafting on the " normal precipitin reaction " were deemed expedient. The chemical nature of the reaction has been dealt with by Chester and Whitaker (1933). The present paper deals with that phase of the subject in which we have endeavored to answer the question of whether it is possible to demonstrate antibody formation in plants by grafting and subsequent testing by the " precipitin reaction " as heretofore employed.

MATERIAL AND METHODS

The experimental plants consisted of representatives of 10 genera and 24 species of the Solanaceae, as follows: *Atropa Belladonna* L., *Browallia viscosa* HBK., *Capsicum frutescens* L., *Datura innoxia* Hort., *D. Metel* L., *D. ferox* Hort., *D. Wrightii* Hort., *Lycium chinense* Mill., *Lycopersicum cerasiforme* Alef., *L. esculentum* Mill., *L. pimpinellifolium* Mill., *Nicotiana Rusbyi* Britt., *N. Tabacum* L., *N. rustica* L., *N. paniculata* L., *N. glauca* Grah., *N. tomentosa* R. & P., *Physalis Peruviana* L., *Petunia violaceae* Lindl., *Solanum nigrum* L., *S. guineense* Hort., *S. Capsicastrum* Link., *S. Melongena* L. These plants were made available to the authors through the courtesy of Professor E. M. East.

The simple whip graft was found to be a very satisfactory method of grafting for our purposes, and for that reason it was used exclusively in the work. The percentage of successful combinations is materially increased if young, succulent, rapidly growing plants are selected for grafting in place of the more mature plants. Stems of the plants selected for grafting should be of approximately the same size. Attention to this detail facilitates the ease with which the graft unions can be bound and insures intimate contact of the cambiums of stock and scion. The stem to be grafted is placed in a special mitre box and then severed by means of a sharp razor blade. The mitre box is used so that the stems can be easily and quickly severed; furthermore, they are cut at the same angle, so that it is comparatively simple to fit the stock and scion together. The union is then firmly bound with raffia. The grafted plants are placed in a warm, moist environment immediately after grafting and allowed to remain there for at least two or three days.

Regarding the care of the grafts after the union has been established, it has been our experience that it is unnecessary to maintain the equality be-

tween the mass of green stock tissues and the mass of green scion tissues which Kostoff believed to be necessary in intergeneric grafts (1929). In some of the most vigorous and healthy of our intergeneric combinations the stock did not have any foliage. Moreover, the junior writer found the same to be true in intergeneric grafts of the Oleaceae, and the same has been observed in many other intergeneric grafts in horticultural propagation.

In making the precipitin tests the method described by Chester (1932a) was used. He found that extracts of dried leaves give results equivalent to those with extracts of fresh material. Several experiments to check this point have proven its reliability. The use of dried material has certain advantages. Thus the extracts can be more readily cleared than extracts of fresh material and, furthermore, the dried material can be stored without deterioration until it is convenient to use it. The use of the dried material also permits a more accurate controlling of the experiments. By retaining a sample of each graft partner at the time of grafting, we were able to make a direct comparison of the " normal precipitin reaction " and the " acquired precipitin reaction " under identical experimental conditions and from identical plants. The material was air-dried for several days, then powdered and extracted with distilled water for a period of 24 hours at 2° C. A solution of one part of dry material in 10 parts of distilled water was used. Other solvents were not tried for the reason that the reactions noted by Kostoff (1929) occurred in distilled water, and it was felt desirable to follow in all essentials the technique used by this worker.

The powdered material for making the extracts was placed in centrifuge tubes and allowed to extract over night under the conditions stated above. The tests were made the following day. The material was centrifuged for 5–10 minutes, and the clear supernatant extract was drawn off by means of a fine-bore pipette and tested in the manner described by Chester (1932a). The strength of the reaction was recorded at intervals of 1, 5, 10, 20, 30, and 40 minutes. In nearly all cases the reaction had reached a maximum at the expiration of 20 minutes, and after this time the precipitate began to diffuse slowly through both liquids, with a consequent blurring or destruction of the ring. The strength of the reaction was scored according to the method employed by Kostoff and Chester.

EXPERIMENTAL RESULTS

In this work, as previously stated, we have attempted to answer the question of whether antibody formation in plants and the resulting acquired immunity can be demonstrated by the " precipitin reaction." To secure an adequate answer to this question, we have performed several types of experiments calculated to give quantitative data on the problem.

By the method of grafting, explained in the previous section, and subsequent testing, 65 individual graft combinations have been tested at intervals of from three weeks to three months after grafting. All possible combina-

30

tions of normal and grafted extracts were tested. An experiment in which *Datura Metel* was used as a scion on a *Lycopersicum pimpinellifolium* stock will serve as an example, illustrating the tests made with the four extracts of each graft combination.

$$D. \text{ } Metel \text{ (normal) } + L. \text{ } pimpinellifolium \text{ (normal) } = +3$$
$$D. \text{ } Metel \text{ (normal) } + L. \text{ } pimpinellifolium \text{ (grafted) } = +3$$
$$D. \text{ } Metel \text{ (grafted) } + L. \text{ } pimpinellifolium \text{ (normal) } = +3$$
$$D. \text{ } Metel \text{ (grafted) } + L. \text{ } pimpinellifolium \text{ (grafted) } = +3$$

The results of the experiments on this phase of the work are summarized and presented in table 1. For convenience the experiments in which the scions are representatives of a single genus have been grouped together. In the table " normal " refers to the " normal precipitin reaction " and " grafted " to the reaction of stock and scion after grafting. According to their appearance at the time the tests were made, the plants were arbitrarily placed in four classes: the plants making the most vigorous growth were classed as " healthy "; the remainder were grouped in descending order as " good," " fair," and " poor "—" poor " indicating a plant that was very sickly and barely alive. It may be added at this point that with few exceptions there was no correlation between the strength of reaction and the health of plants.

An inspection of table 1 shows that in none of the cases recorded is there a significant rise in the " precipitin reaction " after grafting. There are several cases in which the reaction varies slightly in value—i.e., from $+3$ (normal) to $+4$ (grafted). The first experiment in which *Nicotiana Rusbyi* was used as a scion on *Nicotiana rustica* is illustrative of a case where a slight increase in reaction could be scored after grafting. This rise is not considered significant, because experimental errors may be as great as this difference of values. Furthermore, other experiments on the same combination have borne out the fact that there is not a consistent increase in the " precipitin reaction " of this combination after grafting. In several cases there has been a slight drop in the " precipitin reaction " after grafting (e.g., *Nicotiana Tabacum* grafted on *Datura Wrightii*). These slight decreases are not considered significant for the same reasons given where slight increases were observed.

Attention should be called to the fact that in not a single instance under observation was there a reaction after grafting where a negative reaction had been recorded previous to grafting. This is a point which the authors believe should be strongly stressed for the reason that, if reactions of this nature were frequently encountered, the evidence for an immunological interpretation of the " precipitin reaction " would be considerably strengthened, since the acquired reactions in such cases would not be susceptible to error due to the presence of normal " pseudo-reactions."

The writers have been able to identify four different types of reactions as being responsible for the " normal precipitin reaction " in the material they have investigated (Chester and Whitaker, 1933). These reactions have been

TABLE 1

Graft combinations		Reaction		Time elapsed (in days)	Appearance
Scion	Stock	Normal	Grafted		
Browallia viscosa ......*Solanum Capsicastrum*		2	1	36	Healthy
Browallia viscosa ......*Lycopersicum pimpinell.*		3	2	31	Healthy
Datura ferox ....*Atropa Belladonna*		–	–	44	Healthy
Datura ferox ....*Solanum Capsicastrum*		1	1	41	Fair
Datura innoxia ....*Solanum tuberosum*		2	2	22	Fair
Datura innoxia ....*Solanum tuberosum*		2	2	34	Fair
Datura Metel ....*Lycopersicum pimpinell.*		3	3	41	Fair
Datura Wrightii ....*Lycopersicum pimpinell.*		3	3	38	Good
Lycopersicum ceras. ....*Atropa Belladonna*		3	3	27	Healthy
Lycopersicum ceras. ....*Atropa Belladonna*		3	3	45	Healthy
Lycopersicum esculen. ..*Lycium chinense*		–	–	29	Fair
Nicotiana glauca ....*Capsicum frutescens*		–	–	30	Healthy
Nicotiana glauca ....*Nicotiana Tabacum*		–	–	18	Healthy
Nicotiana paniculata ....*Solanum guineense*		–	–	30	Healthy
Nicotiana paniculata ....*Nicotiana Langsdorffii*		–	–	44	Healthy
Nicotiana paniculata ....*Nicotiana Tabacum*		–	–	43	Healthy
Nicotiana Rusbyi ......*Nicotiana rustica*		3	4	25	Healthy
Nicotiana Rusbyi ......*Nicotiana rustica*		3	3	31	Healthy
Nicotiana rustica ....*Nicotiana Rusbyi*		2	2	32	Healthy
Nicotiana rustica ....*Nicotiana paniculata*		–	–	17	Healthy
Nicotiana rustica ....*Nicotiana Tabacum*		–	–	26	Healthy
Nicotiana rustica ....*Nicotiana Tabacum*		–	–	30	Healthy
Nicotiana rustica ....*Petunia violaceae*		–	–	32	Fair
Nicotiana suaveolens ...*Nicotiana Tabacum*		–	–	27	Healthy
Nicotiana Tabacum ....*Atropa Belladonna*		3	3	30	Fair
Nicotiana Tabacum ....*Solanum guineense*		–	–	27	Healthy
Nicotiana Tabacum ....*Solanum tuberosum*		2	1	29	Healthy
Nicotiana Tabacum ....*Datura Wrightii*		2	1	35	Fair
Physalis Peruviana .....*Solanum guineense*		3	3	43	Fair
Physalis Peruviana .....*Solanum nigrum*		–	–	60	Poor
Physalis Peruviana .....*Solanum nigrum*		–	–	49	Fair
Physalis Peruviana .....*Nicotiana Tabacum*		3	3	31	Good
Physalis Peruviana .....*Nicotiana Tabacum*		3	3	30	Fair
Petunia violaceae ......*Atropa Belladonna*		4	4	17	Healthy
Petunia violaceae ......*Atropa Belladonna*		3	3	36	Healthy
Petunia violaceae ......*Solanum guineense*		–	–	24	Good
Petunia violaceae ......*Solanum guineense*		–	–	37	Good
Petunia violaceae ......*Solanum nigrum*		–	–	20	Good
Solanum guineense .....*Petunia violaceae*		–	–	17	Good
Solanum guineense .....*Nicotiana Tabacum*		–	–	16	Good
Solanum guineense .....*Nicotiana Tabacum*		–	–	20	Healthy
Solanum guineense .....*Lycopersicum cerasiforme*		–	–	68	Healthy
Solanum Melongena ....*Lycopersicum pimpinell.*		–	–	29	Healthy
Solanum Melongena ....*Physalis Peruviana*		2	2	46	Good
Solanum nigrum ....*Physalis Peruviana*		–	–	38	Poor
Solanum tuberosum ....*Atropa Belladonna*		–	–	21	Fair
Solanum tuberosum ....*Lycopersicum cerasiforme*		3	1	45	Healthy
Solanum tuberosum ....*Solanum guineense*		–	–	38	Healthy
Solanum tuberosum ....*Datura innoxia*		2	2	44	Poor
Solanum tuberosum ....*Physalis Peruviana*		–	–	59	Poor
Solanum tuberosum ....*Nicotiana rustica*		2	2	29	Fair
Solanum tuberosum ....*Nicotiana suaveolens*		1	1	22	Healthy
Solanum tuberosum ....*Nicotiana Tabacum*		–	–	17	Healthy
Solanum tuberosum ....*Datura Wrightii*		–	–	28	Fair

designated, respectively, as the calcium-oxalate reaction, *AB* reaction, *MN* reaction, and *XY* reaction, and details as to their nature are given in the work cited above. An analysis of the reactions of the various graft combinations should yield some interesting information regarding the type of reaction dealt with in the grafted plants. By a comparison of the data in table 1 with those of table XXV of Chester and Whitaker (1933), one finds that 18 of the positive reactions of the grafted plants involve the calcium-oxalate reaction (table 2). These are simple inorganic reactions caused by the presence of calcium ion in one extract and oxalate ion in the other, producing a precipitate of insoluble calcium oxalate.

TABLE 2. *Graft combinations involving the calcium-oxalate reaction*

Calcium ions absent Oxalate ions present	Reactions		Calcium ions present Oxalate ions absent
	Normal	Grafted	
Atropa Belladonna 4	4		*Petunia violaceae*
Atropa Belladonna 3	3		*Petunia violaceae*
Atropa Belladonna 3	3		*Nicotiana Tabacum*
Atropa Belladonna 3	3		*Lycopersicum cerasiforme*
Atropa Belladonna 4	3		*Solanum nigrum*
Datura ferox 1	1		*Solanum Capsicastrum*
Datura Metel 3	3		*Lycopersicum pimpinellifolium*
Physalis Peruviana 3	2		*Nicotiana Tabacum*
Physalis Peruviana 3	3		*Solanum nigrum*
Physalis Peruviana 3	3		*Solanum nigrum*
Physalis Peruviana 3	3		*Solanum guineense*
Physalis Peruviana 2	2		*Solanum Melongena*
Solanum tuberosum 3	1		*Lycopersicum cerasiforme*
Solanum tuberosum 1	1		*Nicotiana suaveolens*
Solanum tuberosum 2	1		*Nicotiana Tabacum*
Solanum tuberosum 2	2		*Nicotiana rustica*

The five remaining positive reactions are those in which *Nicotiana Rusbyi* and *Datura Wrightii* are involved. This reaction we have called the *MN* reaction (Chester and Whitaker, 1933). It occurs when extracts of either *N. Rusbyi* or *D. Wrightii* containing the principle *M* are tested against extracts of many of the species of the genus *Nicotiana* which contain the principle *N*. Combinations in which this reaction occurs are listed in table 3.

TABLE 3. *Graft combinations involving the MN reaction*

M absent N present	Reactions		M present N absent
	Normal	Grafted	
Nicotiana rustica 3	4		*Nicotiana Rusbyi*
Nicotiana rustica 3	3		*Nicotiana Rusbyi*
Nicotiana rustica 2	2		*Nicotiana Rusbyi*
Lycopersicum cerasiforme 3	3		*Datura Wrightii*
Nicotiana Tabacum 2	1		*Datura Wrightii*

It is thus possible to account for all the positive reactions observed in table 1 by these two types of reaction. It is also evident from tables 2 and 3 that the majority of the positive reactions reported are those in which the

calcium-oxalate reaction is responsible (79 per cent), while the remaining positive reactions involve the M and N principles and are responsible for 21 per cent of all the positive reactions.

There are two exceptions which should be noted. These are the reactions between extracts of *Browallia viscosa* and *Solanum Capsicastrum* and between *Solanum tuberosum* and *Datura innoxia* (table 1). There is the possibility that the former reaction was due to auto-precipitation, for the reason that extracts of *B. viscosa* are very opalescent, making reactions in which this extract participates extremely hard to score. Concerning the reaction between *S. tuberosum* and *D. innoxia*, the plants of *S. tuberosum* were suffering from insect injury both before and after grafting. This may have effected a disturbance in the calcium-oxalate metabolism of the plant sufficient to cause the positive reaction. There is some evidence for this view, since Chester (1931) found that in the morbid processes attending the slow dying of the leaves of either graft-blighted or mechanically injured leaves of the lilac there is an accumulation of oxalate. This would satisfactorily account for the reaction between the insect-injured potato leaves and extracts of *D. innoxia*.

The negative character of many of the tests is of interest and significance from the immunological viewpoint. In many cases, such as *Nicotiana paniculata* grafted on *Solanum nigrum*, there was no " precipitin reaction " either before or after grafting. On the assumption that all the positive reactions in this group are due either to the calcium-oxalate or to the MN combinations, these negative reactions are predictable, since in none of such combinations are both of a pair of precipitating substances present. Moreover, in such combinations as are negative before grafting, all " pseudo-reactions " are eliminated, and if true immunity reactions were present in grafting, they would be most likely to show in such combinations. The fact that all the " acquired reactions " of such plants were also negative points rather definitely toward the non-immunological character of the reactions obtained with the technique employed.

To test further the possibility of demonstrating antibody formation in plants by grafting and subsequent testing by the " precipitin reaction," an experiment was set up in which scions of two different species were grafted on the same stock. The results of an experiment such as this are of interest, since negative results would confirm the findings reported above, while positive results would afford evidence as to the circulation and specificity of immune bodies. Plants of *Solanum tuberosum* having two branches were selected for grafting; upon one branch a scion of *Nicotiana glauca* was grafted, on the other a scion of *Nicotiana Rusbyi*. Two other experiments of the same type were performed. The results of all three of these experiments are brought together in table 4.

This experiment brings out three points: (1) The reaction of each scion towards the stock remained practically the same after grafting as it had been previous to grafting. (2) The reactions of the scions to the stock where two

TABLE 4. *Reactions of graft combinations involving two scions on a single stock*

Graft combinations		Reactions		Time elapsed (days)	Appearance
Stock	Scions	Normal	Grafted		
Solanum tuberosum	*Nicotiana glauca* 1	1		17	Healthy
	Nicotiana Rusbyi .... –	–			
Nicotiana glauca	*Datura ferox* 2	2		23	Healthy
	Nicotiana Rusbyi 1	1			
Solanum guineense	*Datura ferox* 3	3		28	Healthy
	Lycopersicum esculentum .. –	–			

types of reaction are involved (*Datura ferox* and *Nicotiana Rusbyi* grafted on *Nicotiana glauca*) were not influenced by each other. (3) The presence of a reactive scion failed to modify the reactive principles of the stock in such a way as to affect the stock's reaction with the other scion.

These results thus do not support the conception of acquired antibody formation in grafting. This set of experiments is open to the criticism that the experiments were not numerous and that the period of time between grafting and testing was not sufficiently long. However, the writers feel that since all three experiments point in the same direction, and since the reactions in other experiments were not altered when run over a longer period of time, the three conclusions enumerated above seem justified.

In order further to verify the experiments reported above and to gain evidence regarding the circulation and specificity of antibodies in the graft union, we have employed a system of double grafting. A scion of *Datura Metel* was grafted on *Lycopersicum esculentum;* two weeks later a scion of *Solanum Melongena* was grafted on the original *Datura Metel* scion. In their "normal precipitin" relationships, *S. Melongena* and *L. esculentum* are negative to each other; both are positive with *D. Metel.* The plants were found to exhibit the same relationship when tested 27 days later, if we are to judge by the "precipitin reaction."

In the experiment described above we were dealing with the calcium-oxalate reaction. A similar scheme, but substituting plants of *Nicotiana Rusbyi, Solanum tuberosum,* and *Nicotiana glauca* in the order named, to test the *MN* reaction under the same conditions was carried out. *N. Rusbyi* and *S. tuberosum* are negative to each other, but both are positive with extracts of *N. glauca.* Twenty-five days after the grafts were made, the tests of the extracts of the grafted plants indicated that substantially the same relationships existed at the expiration of this period.

These experiments in double grafting thus confirm the experiments described above in their failure to detect changes in the "precipitin reaction" due to grafting, while they offer no evidence for the circulation or specificity

of the reactive substances dealt with. The number of plants we have worked with has been small, but the results are significant when one considers that the trend of both experiments is in one direction.

The question may be asked whether or not acquired immunity in plants (measured by the " precipitin reaction ") is a temporary phenomenon undergoing a rapid increase immediately after grafting and then going through an equally rapid decrease after reaching a maximum. If this were the situation, we would not have been able to detect it in our previous experiments for the reason that in none of them were the grafted plants tested before a period of at least three weeks had elapsed. To secure adequate information on this point, we planned an experiment in which 6 scions of *Nicotiana Rusbyi* were grafted on 6 plants of *Nicotiana rustica* on the same day. The following week the procedure was repeated, using the same number of plants. Each plant was tested at intervals of two weeks over a period of six weeks for the presence of "acquired precipitins." Of the first set of plants, 5 unions were successful; in the second set only 4 survived. The nine plants were tested at the intervals stated above and the results are presented in table 5.

TABLE 5. *Nicotiana Rusbyi grafted on Nicotiana rustica*

Plant no.	Normal	Reactions		
		2 weeks after grafting	4 weeks after grafting	6 weeks after grafting
1	2	2	2	2
3	3	2	2	2
4	2	2	1	2
5	1	1	1	1
6	2	2	2	2
8	1	2	1	1
9	2	2	2	2
11	2	2	2	2
12	1	1	1	1

This experiment shows quite clearly that of the 9 plants tested not a single plant produced reactions indicative of acquired immunity. During a period of two weeks after grafting there were no indications of a sharp rise or decline in the " precipitin reaction " as described by Kostoff (1928–1931); the reactions of single plants during this six weeks' period were fairly consistent, only slight increases or decreases being observed, and these cannot be regarded as significant.

DISCUSSION

In our experimental work we have attempted in several ways to demonstrate antibody formation in plants by the use of the " precipitin reaction." The evidence has been negative from every line of approach we have undertaken. We have not observed a single clear-cut case of an " acquired precipitin reaction " in a total of over 70 experiments to test the question.

It must be distinctly understood that we do not claim to have shown that antibody formation in plants is not possible, but we do state that with the technique and species used it was impossible to demonstrate acquired immunity in plants. There are four lines of evidence from which this statement can be drawn:

(1) The results from testing 65 individual graft combinations involving 10 genera and 24 species of solanaceous plants did not give the slightest indication of "acquired precipitin reactions." The grafted plants were tested from periods of three weeks to three months after grafting, and the results were very uniform, with every indication that the reaction was practically the same after grafting as it was previous to grafting.

(2) The fact that of our positive reactions 79 per cent were reactions between calcium and oxalate ions places considerable doubt on the immunological significance of the "normal precipitin reaction" in plants. It would be unsound to give an immunological interpretation of a simple inorganic reaction such as this. Moreover, we have shown elsewhere that the methods employed by previous workers in this field fail to eliminate simple inorganic precipitates of this nature. The remaining 21 per cent of the positive reactions were due to the MN combination. This reaction has been shown to be caused by a substance M (probably a protein) interacting with a less complex organic substance N. From this evidence and from the fact that "acquired precipitin reactions" were not demonstrable in the grafts involving the MN combination, one should be rather hesitant in attributing immunological significance to this particular reaction. Certainly it is not justified in the light of the present work.

(3) The experiments with two scions on one stock and with regrafting are confirmatory of the conclusions stated above and lend no support to the immunological interpretation of the graft reactions.

(4) In experiments where the MN reaction was involved there was no indication that acquirement of precipitins takes place at any period between two and six weeks after grafting.

These four phases of our experimentation afford sound evidence to support our statement made previously that antibody formation in plants has not been demonstrated by the "precipitin reaction."

The essential feature of the work of Kostoff (1929) was the reported occurrence of "acquired precipitins" in five combinations of grafted plants. The combinations in which the "precipitin" potency was found to increase are listed below:

(1) *Solanum tuberosum* grafted on *Solanum Lycopersicum.*
(2) *Lycium Barbarum* grafted on *Solanum Lycopersicum.*
(3) *Nicotiana Rusbyi* grafted on *Nicotiana rustica.*
(4) *Nicotiana glauca* grafted on *Capsicum pyramidale.*
(5) *Nicotiana Sanderae* grafted on *Datura ferox.*

We have repeated the first four of these combinations, using species and, for the most part, stock identical with those with which Kostoff secured his positive results.   In place of *Capsicum pyramidale* we have used *Capsicum frutescens,* but our data show that these two species are identical in their reactions.   The results we have secured give no indication that the " precipitin reaction " capacity was increased after grafting.   Our technique except for minor variations was the same as that used by Kostoff, so that differences in the technique are not sufficient to account for the divergence in the results that occurs.   Furthermore, we have shown that it would be most improbable to expect an increase in the " precipitin reaction," for the reason that a large majority of these reactions are of simple inorganic nature.   Of the five combinations reported above, three of them, we have found, are due to the calcium-oxalate reaction (*S. tuberosum* on *N. rustica, N. Sanderae* on *D. ferox,* and *N. glauca* on *C. pyramidale*), one is due to the *MN* reaction (*N. Rusbyi* on *N. rustica*), and one is completely negative (*Lycium Barbarum* on *L. esculentum*).

Silberschmidt (1931) has published a lengthy paper dealing with the specificity of the " normal precipitin reaction " and the methods of pre-extraction and solution.   In connection with this work he has reported experiments in which he has tested grafted solanaceous plants for acquirement of precipitins.   In only one case has he secured positive results—namely, when *Nicotiana glutinosa* was used as a scion on *Datura sanguinea.*   In this case he states that there is a possibility that his results can be explained by antibody formation, although he is very skeptical about the matter.   There is also the possibility that in this case the investigator was dealing with the calcium-oxalate reaction, since we have shown (1933) that this reaction cannot be eliminated by any of the methods of pre-extraction and solution employed by Silberschmidt.   Our data show that *Datura* species usually contain an excess of oxalate ions and *Nicotiana* species usually contain an excess of calcium ions; so there is the possibility that this explanation would be sufficient to cover the point in question.

The interpretation of agglutinating plastids, disturbed meiotic divisions, abnormal corollas, and tumor formation in grafted plants as due to antigen-antibody reactions (Kostoff, 1929, 1930) is not supported by the present work with the " precipitin reaction," and most of Kostoff's immunological interpretations in this field pivot on his " precipitin reactions," which in his studies are of fundamental importance in supporting his thesis.

SUMMARY

1. Seventy-five graft combinations consisting of representatives of 10 genera and 24 species of the Solanaceae have been tested for the presence of " acquired precipitins " after grafting.   In this series of experiments not a single case has been discovered in which the " precipitin reaction " was appreciably greater after grafting than it was previous to grafting.

31

2. An analysis of the positive reactions included in this series of graft combinations indicates that two different types of reactions are concerned. The calcium-oxalate reaction is involved in 79 per cent of the positive cases and the *MN* reaction, previously described by the authors, in 21 per cent of the cases. It has further been shown that there is no evidence for attributing immunological significance to either of these reactions.

3. When two scions are grafted on a single stock, either in parallel or in series, the reaction of each scion to the stock does not undergo significant changes when compared with the reactions previous to grafting. This fact can be considered as another point unfavorable to the interpretation of the "precipitin reaction" as an antigen-antibody reaction.

4. Experimental evidence from the *Nicotiana Rusbyi-Nicotiana rustica* graft combination has shown that there is no significant increase in the "precipitin reaction" of this combination from a period 2 weeks after grafting to 6 weeks after grafting.

5. The experiments reported above thus give no indication that as a result of grafting there is an acquired immunity of stock to scion or of scion to stock demonstrable by the "precipitin" technique employed up to the present.

The writers wish to express their appreciation to Professor E. M. East for suggestions, helpful advice, and criticism.

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STRUCTURE AND GERMINATION OF TOBACCO SEED AND THE DEVELOPMENTAL ANATOMY OF THE SEEDLING PLANT

GEORGE S. AVERY, JR.

INTRODUCTION

The genus *Nicotiana* (Solanaceae) is composed of some 50 or more species, according to Garner (1927), and possibly as many as 70 species, according to Setchell (1921) and East (1928). For purposes of classification the genus was divided into four sections by Don (1838): Tabacum, Rustica, Petunioides, and Polidiclia. These were accepted by succeeding authors, including Comes (1899), but East (1912) and Setchell (1912) both agree that the one species and its variety in the section Polidiclia belong in Petunioides. Later, East (1928) concludes that the Petunioides section is not justified from the genetic evidence. He states: " There are a number of genetic centers which may possibly be made the basis for various generic subdivisions when our information is more complete." Chromosome numbers in the genus are diverse, the haploid numbers of some 33 different species thus far determined being 8 or 9, 10, 11, 12, 16, and 24 (East, 1928; Gaiser, 1930), appearing in no way related to the present grouping into sections.

It is generally conceded that the genus is of New World origin, Central America and northwestern South America being centers of distribution, according to DeCandolle (1884), Vavilov (1926, 1931), and East (1928), though *N. suaveolens* Lehm. is reported from Australia and three other species which may be merely varieties of the latter are listed from the region of the Malay peninsula (East, 1928). Some species are herbaceous annuals, others perennials, and some even shrubby or subarborescent (four such species are distinguished by Goodspeed, 1932, including *N. Wigandioides* Koch.), or arborescent (*N. glauca* Graham). Certain species produce showy garden flowers, while still others are valued for their leaves. Among the latter is *Nicotiana tabacum* L., our common tobacco of commerce, sharing its importance to some extent with *N. rustica* L. (Bailey, 1916).

While there is considerable diversity of opinion as to who it was that first took tobacco or tobacco seed to Europe, there does seem to be a general agreement that it took place in the first half of the sixteenth century. Since its introduction into Europe four centuries ago it has developed into one of the most important plants of commerce, statistics for the year 1930 indicating a production of over one and a half billion pounds (*International Yearbook*

of Agriculture, p. 210, 211), Soviet Russia and several smaller countries not included. The increase in its culture has been marked in the past decade. Diseases take a heavy toll in some regions.

An ideal study in the developmental anatomy of a seed plant should perhaps include four periods in gross development: The *first* is concerned with the embryo plant—i.e., its development from the zygote to the mature embryo in the seed. This particular stage is determinate, in that it has a definite beginning and end. The *second* period is concerned with the enlargement of these first-formed parts, ending in the usual category of the seedling plant. The *third* period consists, at first, of enlargement which takes place as growth is accelerated (in tobacco it includes the stages in which the first ten to fifteen leaves develop), and this grades insensibly into the condition in which are developed all vegetative structures typical of the adult plant in size and character. This period of vegetative development may extend over a longer time than any of the others and is often marked by considerable vegetative plasticity, due to extremes of soil moisture, light, or other conditions. It is a period during which, in the growing plant, "continued embryology holds sway with its successive origination of new organs" (Bower, p. 315). The *fourth* is a period which occurs after a certain physiological balance has become established within the plant, resulting in the development of parts leading to and including flowering and fruiting.

The earlier stages in the embryogeny of tobacco have been worked out by Souèges (1920), to illustrate the origin of the dermatogen, plerome, and periblem, and the study here presented is confined to the development of the seedling plant and the beginning of the period of rapid enlargement which follows, chiefly as regards the hypocotyledonary axis.

The results that follow, though little more than confirmatory of many preceding investigations on seedling anatomy, attempt to give more detailed information on the developmental side of what has become one of our most important economic plants. The present paper represents the first of a series of studies on the structure and development of *Nicotiana tabacum* L.

MATERIALS AND METHODS

The greater part of the seeds and seedlings of tobacco (*Nicotiana tabacum*) used in this experiment were of the Cash variety, although Havana Seed and Cuban Shade varieties were used as additional material. Less extensive observations were made on *N. Langsdorffii* Weinm., *N. glutinosa* L., and *N. rustica,* each species representing a different chromosome group. In germinating the seeds no particular attention was paid to temperature, though Haberlandt reports an optimum of 27° C., with the minimum and maximum at 15° and 31°-33° C., respectively. No seed treatment nor special precaution was taken with regard to light, but it seems well to touch on these points because of their relation to germination.

Goodspeed (1913) has shown that treatment of seed from 10 to 12

minutes with 80 per cent H_2SO_4 and washing for a short period markedly increases the percentage of germination for several *Nicotiana* species. While the necessity of light for germination is a problem on which there are many diverse data and subsequent conclusions, Honing (1916, 1926, 1930) finds that such varieties as Cuban Shade, Connecticut Broadleaf, and many of the varieties grown in the Carolinas and Virginia show only a small percentage of germination in the absence of light as compared with its presence (0–10 per cent), while Havana Seed in darkness shows 41–50 per cent as good germination in darkness as in light. *N. rustica* under similar circumstances shows 71–80 per cent as good germination. All figures were based on observations at the end of seven days. Goodspeed (1919) reports that " there is no doubt that the seed of five representative types of *Nicotiana tabacum* and of five varieties of *N. rustica* will germinate readily in darkness." Numerous other papers deal with the subject.

The material taken for microscopic study was fixed in formal-acetic-alcohol, imbedded by the paraffin method, sectioned at 10–12 microns, and stained with safranin, being counterstained with Delafield's haematoxylin or fast green.

In the studies on seeds as well as seedlings, serial sections were cut both longitudinally and transversely.

THE SEED

The seeds of tobacco are very small, egg-shaped though somewhat flattened, and with a prominent raphe along one side, ending in the projecting hilum at the small end of the seed (fig. 1, *A*). They have a finely reticulated surface and are dark brown in color. Though the seeds vary in size according to variety, they average, according to Kondo (1921), 0.75 mm. long. 0.53 mm. broad, and 0.47 mm. thick, a thousand weighing 0.08 gram.

The protective seed coat (not illustrated in detail) includes a prominent epidermis. Both layers of the double inner wall are cutinized and the inner layer is slightly lignified. The outer walls are thin, of cellulose with some pectic material, and are lightly cutinized. The fact that the thin outer walls bend inward at maturity accounts for the reticulated appearance of the seed. The subepidermal layers, usually three in number, are composed of thin-walled parenchyma cells which are crushed at maturity. It is necessary to study an immature seed to get the seed coat in full detail, as pointed out by Grintescu (1915) for *N. rustica*. A single layer of nucellar tissue persists just inside the layers of parenchyma, between the latter and the endosperm. Inward from this are some three to five layers of rather thick-walled endosperm cells (fig. 1, *K* and *L*), largely isodiametric, rich in aleurone and oil droplets and densely protoplasmic.

A longitudinal section through the seed (fig. 1, *L*), passing through the plane of symmetry of the straight or slightly curved embryo, shows the latter to be about 0.7 mm. long and surrounded by endosperm. The cotyledons
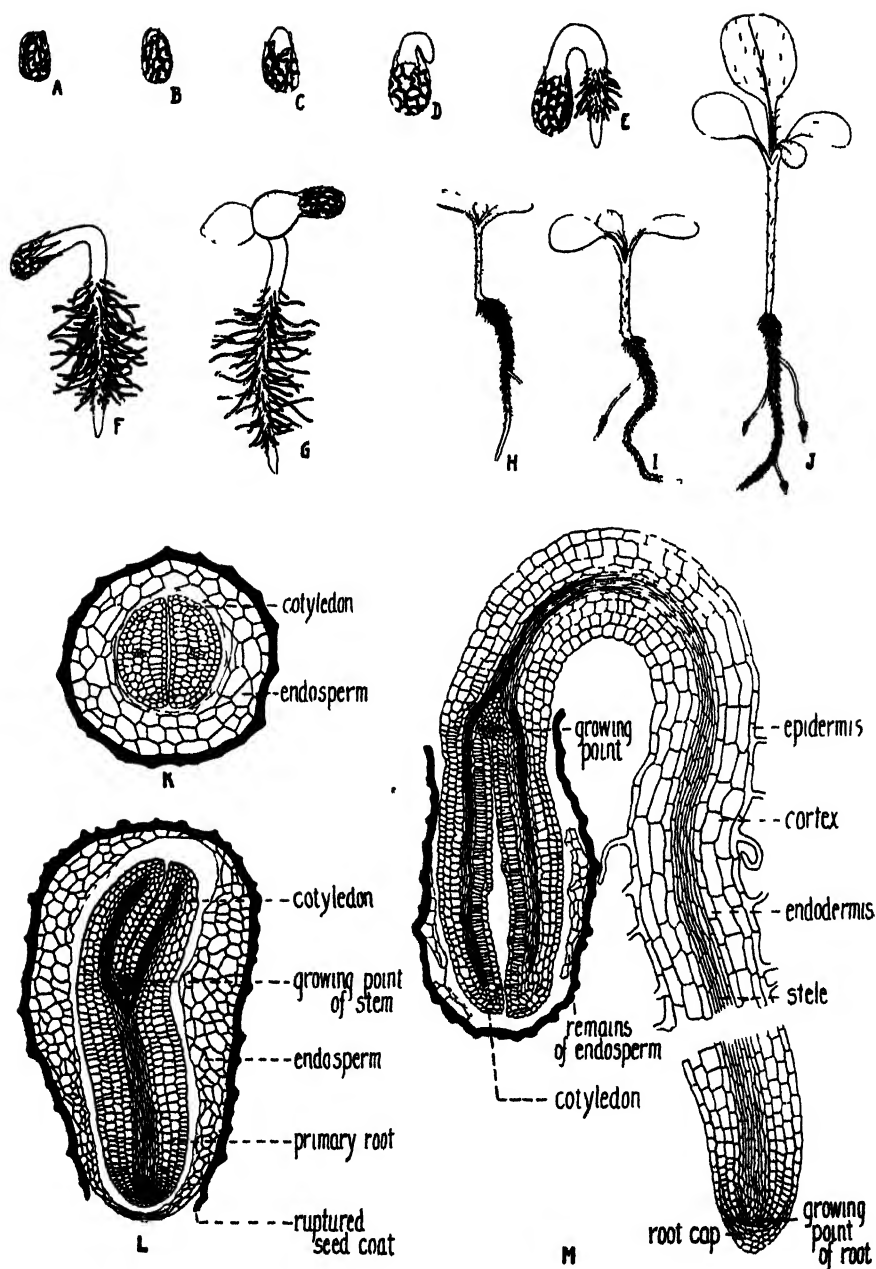
Fig. 1, *A–M*. *A*, dormant seed (×8). *B–D*, stages in germination at the end of six days (×8). *E–G*, same, at the end of nine days (×8). *H–J*, seedling stages at the end of sixteen to eighteen days (×2). *K*, transverse section through dormant seed at the cotyledonary level (×70). *L*, longitudinal-median section through seed just starting to germinate, showing ruptured seed coats (×50); see *B* above. *M*, longitudinal-median section through seedling at the end of nine days (×50).

are six layers of cells in thickness, including upper and lower epidermis (fig. 1, K). Their provascular midrib is well defined, as is the central strand of the hypocotyl, the latter being oriented toward the small end of the seed. In the hypocotyl there are four layers of cells outside the stele, including the epidermis (fig. 1, L and M). It is of interest to note that the mesophyll of the cotyledons is of the same number of cell layers in thickness as the cortex plus the epidermis of the hypocotyl. The embryonic cotyledons are some seventeen upper epidermal cells long, other layers having about the same
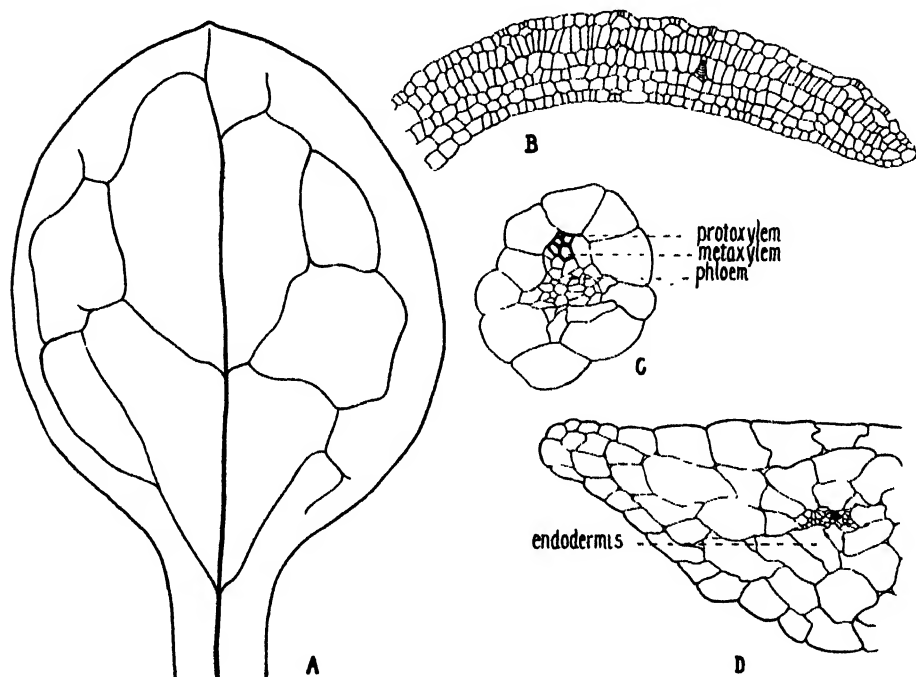
Fig. 2.   *A-D. A*, diagram of mature cotyledon showing entire vascular system ($\times$ 20). *B*, longitudinal section of cotyledon of a seedling nine days old ($\times$ 70); see fig. 1, *G. C*, transverse section through midvein of mature cotyledon, midway between base and tip ($\times$ 150). *D*, transverse section through mature portion of cotyledonary petiole ($\times$ 120).

number, and eighteen upper epidermal cells wide (fig. 1, K and L). Behrens (1892) reports "two procambial strands corresponding to the bundles of the cotyledons" in the hypocotyl. This seems to be the condition only at the upper end as they diverge into the cotyledons. The growing point of the stem, though only slightly developed, is markedly conical in shape.

GERMINATION

Germination is irregular in most cases, as Behrens (1892) pointed out. The first germinations usually take place six to eight days after planting.

The seed coat breaks under the pressure of the developing primary root, near the micropylar end of the seed (fig. 1, B and L). The hypocotyledonary axis continues elongation and in 10–12 days the primary root is several millimeters in length, has developed copious root hairs, and has started to function (fig. 1, C–F). The upper hypocotyl is slightly hairy. During the same period the cotyledons have remained within the seed coats (fig. 1, M), usually withdrawing about the twelfth day, though the old seed coats often adhere to one of them for a few days longer (fig. 1, G). By the fifteenth day the first leaf above the cotyledons is visible, followed by the second on the seventeenth or eighteenth day (fig. 1, H–J). Growth accelerates rapidly from this time on. These stages in germination are discussed below from the point of view of internal development.

ONTOGENY OF THE PRIMARY ROOT

The growing point of the root is developed at the time of differentiation of the embryo, the lower end of the hypocotyledonary axis possessing definite though immature root structure.

The primary root is diarch (fig. 3, A-1), the protoxylem and metaxylem being practically indistinguishable from each other. The primary phloem groups consist of two small masses of parenchyma, one on either side of the xylem arm. The pericycle is clearly distinguishable as the outer layer of the stele and gives rise to a few lateral roots after a period of fourteen to sixteen days (fig. 1, H). The Casparian strip identifies the endodermis, the other two layers of the cortex being composed of considerably enlarged parenchyma cells. The epidermis is thin-walled and gives rise to copious root hairs. The number of cell layers of the cortex corresponds to the number present in the embryo, there being no increase in cell number in transverse section as the cortex enlarges.

ONTOGENY OF THE HYPOCOTYL

The rapid increase in the length of the hypocotyl is due both to cell division and enlargement, *all new cell walls of the primary body being laid down perpendicular to the axis*. The number of cells in a transverse section of the hypocotyledonary axis of the seedling before secondary thickening would, therefore, be the same as the number present in the embryo.

Serial sections up through the axis of a seedling ten days old disclose the internal conditions at this early period of development (fig. 3, A-1 to A-13). It is clear at this stage of development that most of the anatomical root-to-stem change takes place in the region immediately below the cotyledons, from the level shown in figure 3, A-6, to approximately the cotyledonary level (fig. 3, A-13), a distance of about 0.5 mm. This portion of the hypocotyl is still elongating, and the cotyledonary traces of annular and spiral elements are clearly discernible (fig. 3, C). The protoxylem of the trace to the first leaf distal to the cotyledon has differentiated (fig. 3, A-12), though the leaf has
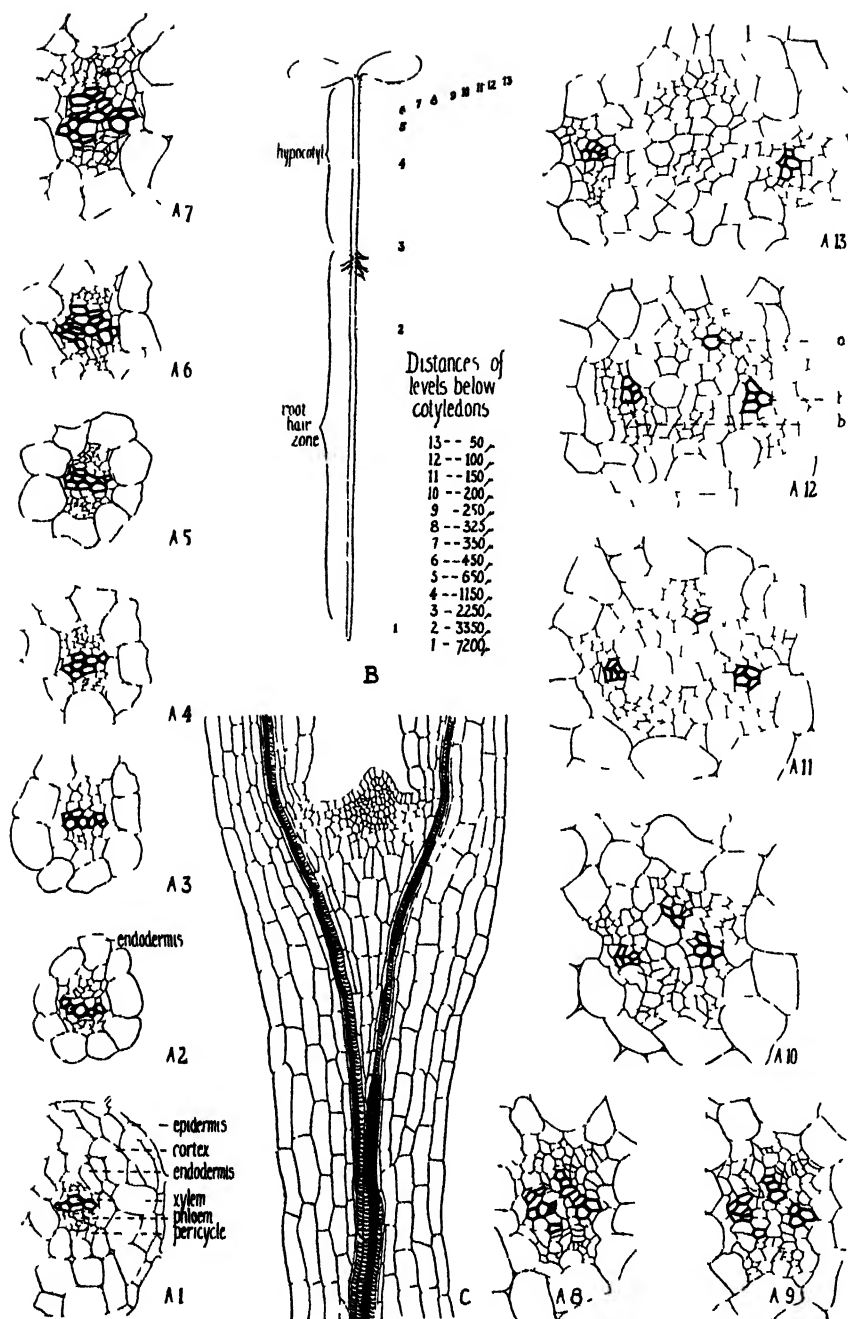
Fig. 3, *A*-1 to *A*-13, *B*, *C*. All figures from seedlings ten days old. *A*-1, transverse section through portion of root at the level indicated in *B*-1 ($\times$ 150). *A*-2 to *A*-13, transverse sections of stele and endodermis at successive levels indicated in *B*-2 to *B*-13 ($\times$ 150). (*A*-12. *a*, xylem of trace to first leaf distal to cotyledon. *b*, xylem of cotyledonary traces.) *B*, sketch of seedling ($\times$ 1½) to show levels at which transverse sections *A*-1 to *A*-13 were taken. *C*, longitudinal-median section through hypocotyl showing transition region, divergence of cotyledonary traces, and growing point of stem.

not yet developed. The only clearly differentiated cells in the stele at this level are the xylem cells of these three traces, though the endodermis is easily identified (fig. 3, *A*-10 to *A*-12). The origin of the pith is evident at level *A*-8, 325 microns below the cotyledons, where the diarch root xylem becomes divided into several groups. The two more prominent groups become the xylem of the cotyledonary traces, the two less prominent ones ultimately supplying the first and second leaves distal to the cotyledons. At the level at which the pith is first evident, each phloem group from the root bifurcates, so that the xylem of each cotyledonary trace is central and is flanked on either side by a phloem group. The phloem on one side of one cotyledonary trace forks again, one of these strands accompanying the xylem destined for the first leaf distal to the cotyledons (fig. 3, *A*-10; fig. 1, *H*), the other remaining with the cotyledonary trace. At the time these phloem changes are taking place, the protoxylem becomes reoriented so that the xylem of each cotyledonary trace is endarch.

The axis of a seedling three to four weeks old is the first to show clearly the relationship between internal and external phloem. At this period of its development the seedling has two to three leaves visible above the cotyledons (fig. 1, *J*). Secondary thickening has just begun. The change from the exarch to the endarch condition of the primary xylem, though difficult to make out because the seedling of tobacco is greatly reduced, takes place in a seedling of this age about 2 mm. below the cotyledonary level and is completed within a distance of approximately 1 mm. The fact that transition takes place less than 0.5 mm. below the cotyledonary level in the younger material indicates that the region immediately below the cotyledons has undergone elongation. With regard to the establishment of the internal phloem, it is evident for the first time in material of this age that strands of external phloem diverge inward approximately 1.4 mm. below the level at which the cotyledons are attached to the axis.

A hypocotyl four to six weeks old, cut in serial transverse sections, shows the relationship of external and internal phloem in its final ramifications (fig. 4, *B*; fig. 5, *A–D*). The secondary xylem is considerably increased in amount. In some material the first two phloem strands enter the pith 180 degrees from each other, being laid down gradually inward through the cotyledonary gaps, the internal condition in each case being established within a perpendicular distance of 60 microns. When the first two strands of internal phloem are oriented in this manner at a level about 1.4 mm. below the cotyledons, they are usually followed by two more strands which become established internally at a level approximately 0.7 mm. below the cotyledons. These also "invade" the cotyledonary gap (opposite side of the cotyledonary trace from the previous "invasion"), and are 180 degrees from each other. Other internal phloem strands may have differentiated in material of this age, in which case they usually connect with the external phloem through the gaps already mentioned or through vascular rays, often immediately

level 4·B 450μ

5·A 690μ

5·B 942μ

5·C 1142μ

5·D 1466μ

5·E 1706μ

5·F

3706μ

A

—level of coty-
ledonary
divergence

---cotyledonary trace

---internal phloem

---pith

---pericycle

-endodermis

cortex

-epidermis

cotyledonary
trace

phloem
xylem
phloem

---cortex

to 1ˢᵗ leaf
distal to cotyledon
pith
internal phloem

to 2ⁿᵈ leaf
distal to cotyledon
external phloem

B
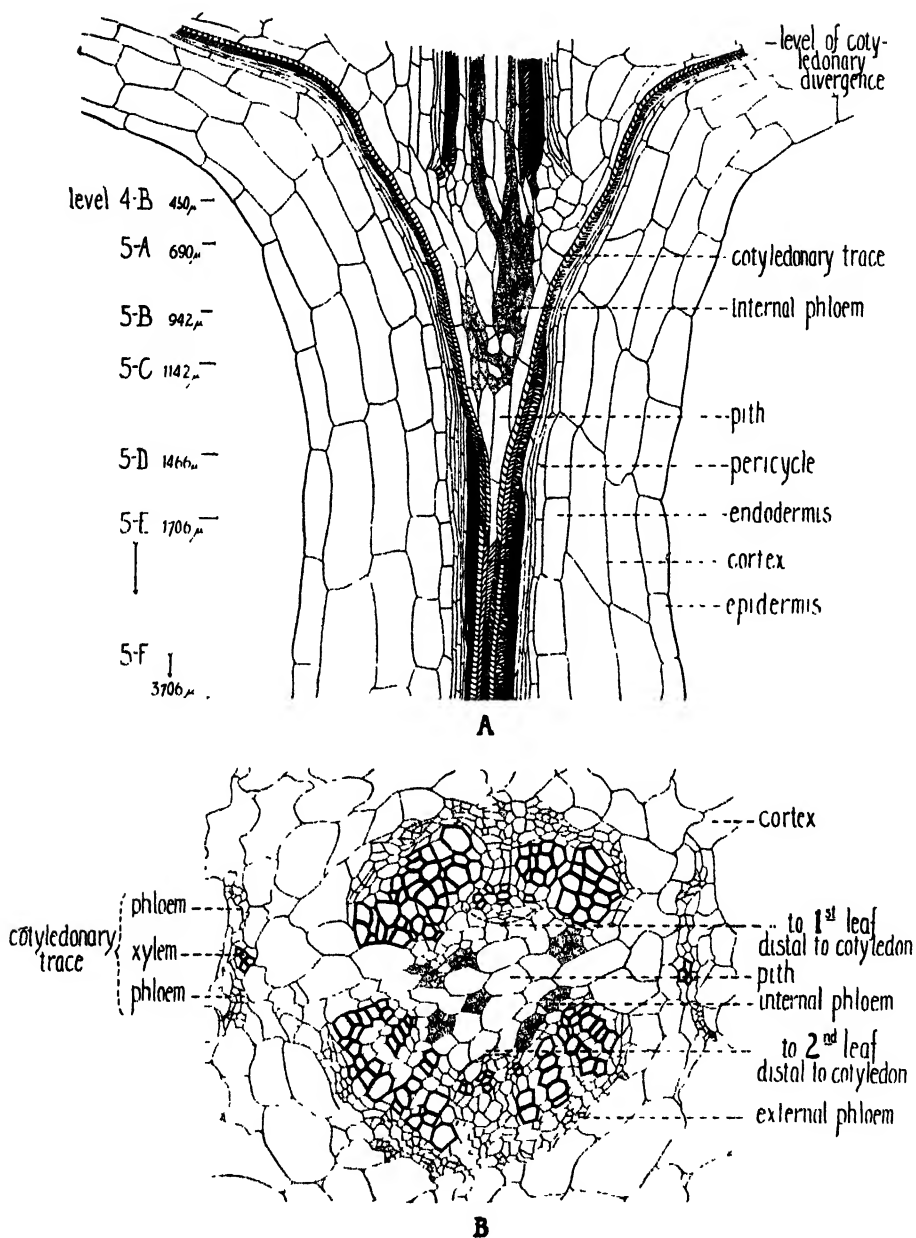
Fig. 4, *A* and *B*. Both figures taken from plants six weeks old. *A*, longitudinal-median section through upper hypocotyl showing orientation of internal phloem, divergence of traces to the cotyledons and the lower end of the first internode (× 50). Levels 4, *B*, and 5, *A-E*, indicate levels at which transverse sections of same age were taken, and refer to figure 4, *B*, and figure 5, *A-E*. Distances below the level of cotyledonary divergence are indicated at each level. *B*, transverse section through portion of the hypocotyl at the level indicated above as 4, *B* (× 140), approximately 450 microns below actual cotyledonary divergence.
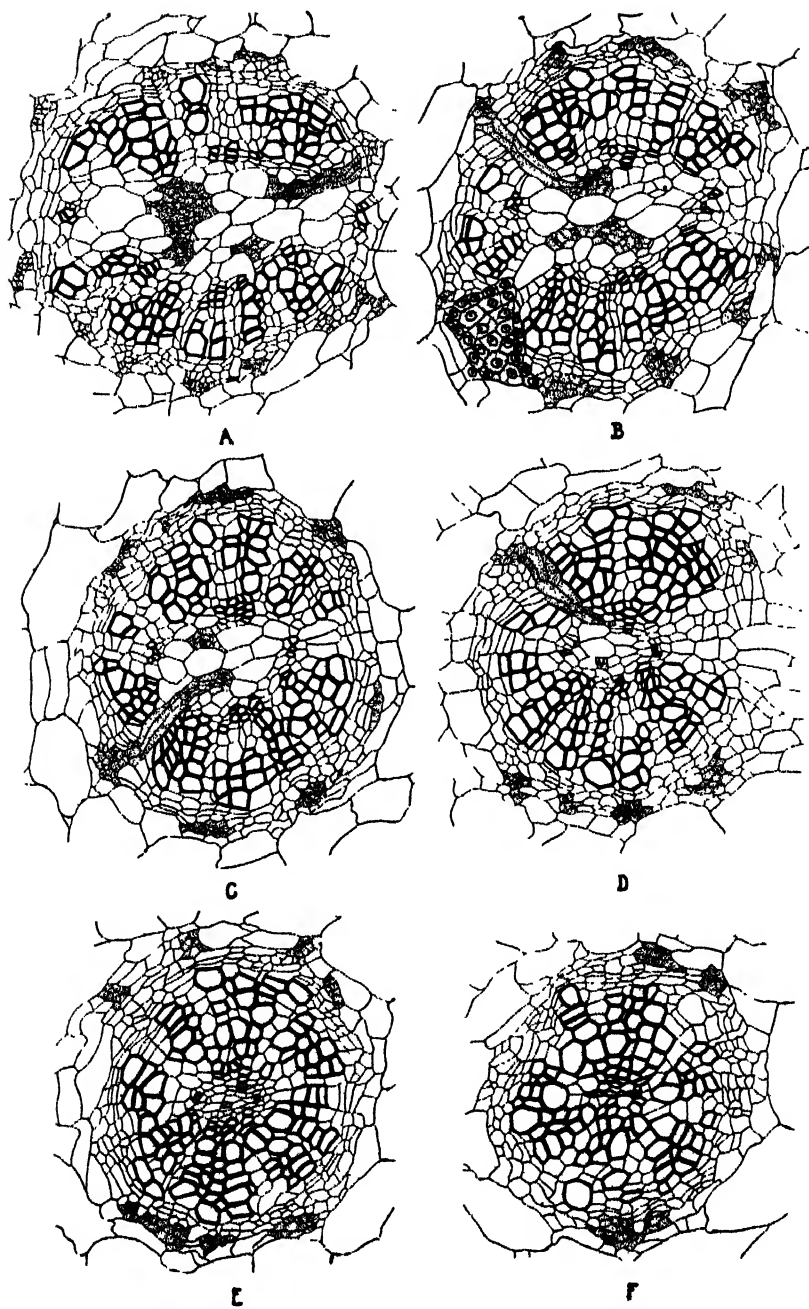
FIG. 5.

beside the trace to the first leaf distal to the cotyledons, well below the level of the leaf gap.

Apparently there is no definite pattern for the establishment of the internal phloem, for it occurs in diverse ways. A common scheme of accomplishment is illustrated in figure 5, *A–D*. In this case, three of the internal phloem groups " enter " the pith from one side of the axis, while the fourth enters from the opposite side. A fifth internal phloem strand, very small in size, ends blindly in the pith (fig. 5, *D* and *E*). As previously indicated, each phloem strand which differentiates inward from the external phloem accomplishes the change within a distance of 60 microns. As further secondary thickening takes place, the connection between internal and external phloem groups is by means of phloem parenchyma laid down by cambium. The internal phloem anastomoses to form something of a phloem plate just below the cotyledonary level (fig. 4, *A*). From four to seven groups of internal phloem usually appear in transverse section at this level.

The root system of the mature tobacco plant is largely adventitious, and such roots are being initiated by the pericycle throughout the hypocotyl of a seedling four to six weeks old (fig. 5, *B*).

The number of epidermal and cortical cells present in a transverse section of the hypocotyl at this stage of development is essentially the same as in the corresponding structure of the embryo, but they are considerably enlarged. There is only a small amount of external phloem present, primary and secondary, and this appears to be largely parenchyma. The secondary xylem elements are scalariform or reticulate in their markings.

ONTOGENY OF THE COTYLEDONS

All new cell walls in the cotyledons (except in provascular regions) are likewise laid down perpendicular to the plane of the upper and lower epidermis. The number of cell layers in a mature cotyledon is, therefore, the same as in an embryonic cotyledon.

Fig. 5, *A–F*. All figures are from serial transverse sections through the hypocotyl of a seedling six weeks old ($\times$ 140). *A*, 240 microns below transverse section shown in fig. 4. *B*, showing internal phloem connected through cotyledonary gap with the external phloem. *B*, 252 microns below *A*. The connecting phloem shown in *A* has joined the external strand. Another internal phloem group is shown connecting with the external phloem. At the lower left is the primordium of an adventitious root. *C*, 200 microns below *B*, showing connecting phloem strand. In the late primary condition this would have appeared as taking place through the cotyledonary gap. The laying down of secondary xylem has made the connecting phloem actually secondary, through a vascular ray. *D*, 324 microns below *C*, showing the last remaining internal group connecting with the external phloem. *E*, 240 microns below *D*, showing a vestige of internal phloem which ends blindly a little lower down. The xylem of the cotyledonary traces is closing in toward the center and the pith is much reduced. *F*, 2 millimeters below *E*, showing pith no longer present. The diarch primary xylem is characteristically root-like, though buried in secondary xylem.

The upper epidermal cells are the first to divide in the cotyledons, and stomata of the upper epidermal layer are well differentiated long before the cotyledons leave the old seed coat. The palisade cells assume their characteristic appearance early, division having started almost simultaneously with the upper epidermis. Growth is predominantly basipetal until the seed coat is shed, expansion then taking place rather evenly throughout the lamina. The petiole continues to elongate for some time.

The cotyledons at the time of emergence average forty-eight upper epidermal cells in length, with other cell layers about as follows: lower epidermis, forty-two; palisade, fifty-six; spongy parenchyma, thirty-seven. Since seventeen cells is the average for each layer in the cotyledons of the dormant embryo, it is obvious that the palisade undergoes the greatest number of divisions, followed by the upper and lower epidermis, and lastly the spongy parenchyma. The latter through meristematic activity is responsible for all vascular bundles of the cotyledon except the midrib, which is already differentiated in the dormant embryo.

At the end of ten to twelve days the primary xylem is mature in the cotyledons as well as in the hypocotyl. In the midvein it consists of annular and spiral (occasionally scalariform) elements, and is distinctly endarch in its development from petiole to tip of cotyledon. Only slender elongate parenchyma cells have been observed in the phloem of the cotyledons.

The emergence of the cotyledons from the seed coat is due to their predominantly basipetal growth. At this time the cells of the upper epidermis increase rapidly in size, thus opening the upper cotyledonary surfaces to the sunlight. The lower epidermal cells are somewhat slow to divide and differentiate, for as long as the cotyledons remain in the seed the lower epidermis acts as an absorptive layer, taking in food from the endosperm (Behrens, 1892).

The mature cotyledons of the Çash variety at the end of their period of expansion are some forty epidermal cells wide and seventy cells long (not including the short petiole), indicating almost twice the number of cell divisions in length as in width. Even in length the number of cells is only four times the number present in the embryo, which indicates a considerable cell enlargement. The greater number of these divisions take place before the cotyledons emerge from the seed coats, as indicated above. The average area of the cotyledons is 14.4 sq. mm., while the average total length of veins per cotyledon is 27.3 mm.

In the petiole of the mature cotyledon (fig. 2, D), the endodermis is clearly differentiated as the bundle sheath and may be distinguished by its Casparian strip. The outer epidermal wall and cuticle of the cotyledon are rather thin, offering little mechanical protection against the invasion of parasitic organisms.

DISCUSSION

Earlier mention has been made of the fact that this work is largely confirmatory of the results of previous studies of seedling plants. Its principal contribution is to give more detailed information on the developmental anatomy of tobacco, one of our most important economic plants.

As to the weight of tobacco seeds, Kondo (1921) reports them as averaging 0.08 g. per thousand, but Berthold (1931) shows that seeds are heavier if some of the flowers are removed from the inflorescence and relatively few capsules are allowed to develop. Seed weight per 1000 in such a case runs as high as 0.0859 g., as against a minimum of 0.0723 g. without trimming. Berthold has also shown that seeds in the center of the inflorescence are heavier.

With regard to the structure of the seed, the findings here are in almost complete agreement with those of de Toni and Paoletti (1891), working on *N. tabacum*, and with those of Grintescu, working on *N. rustica*, while the descriptions and figures of Harz (1885, p. 1020, 1021) and Behrens (1892) are inadequate.

The relatively rapid acceleration in the growth of the seedlings, once started, was noted and measured by Behrens (1892). He found the dry weight of the plant at the end of 20 days to be 38 times its seed weight, indicating a reasonably rapid development for so minute an embryo and small amount of stored food.

From an anatomical point of view, the principal works which have included mention of *Nicotiana* and related genera have been those connected with studies of the origin or merely of the presence of internal phloem and the root-stem transition. Several early studies go into the origin and presence of internal phloem in solanaceous plants; but Gérard (1881), working with *Datura,* was the first to determine that *branches of external phloem were laid down inward to become internal phloem.* Scott (1891) finds evidence in *Ipomoea versicolor* Meissn. which confirms Gérard. The internal phloem of the hypocotyl joins the external phloem of the root, having differentiated outward between the converging protoxylem groups of the cotyledonary trace.

Herail (1885), from a study of developing petioles of tobacco leaves, among other things, concluded that the internal phloem had its origin in pith parenchyma, was not connected with the external phloem, and should be called, therefore, "medullary phloem." He made the point that the internal phloem always has the same structure as the external phloem. Flot (1893), on the other hand, distinguishes the perimedullary zone (the part which borders the woody portion of the bundle on the inside) in which the internal phloem arises, as having its origin in the procambium. He uses tobacco in support of this contention (fig. 10, pl. 4; fig. 15, pl. 6).

Lamounette (1890), in one of the more comprehensive works on internal phloem, reported on tobacco as well as numerous species from genera of

various families with internal phloem. He, with Herail, held that internal phloem is derived from parenchymatous cells adjacent to the procambial ring, and not from the procambium. Because of their interpretation that the internal phloem is of a different origin and is not, therefore, a part of the vascular bundle, they object to the term " bicollateral " proposed earlier by de Bary (1877). Lamounette lists 21 families with internal phloem, and states (p. 214) that he has never observed immediate relations between the internal and external groups. (The fact that one internal phloem strand in tobacco did not join the external phloem is evidence that such a union does not necessarily have to take place, and in some plants may not, as reported by Lee, 1912, p. 737, for *Schizanthus pinnatus*.) According to Lamounette, plants with internal phloem may be placed in two categories: (1) those having internal phloem at the lower end of the hypocotyl, in the immediate neighborhood of the root; (2) those in which the internal phloem appears at the summit of the hypocotyl, at the level of divergence of the cotyledons. He further concludes that the internal phloem of the hypocotyl is independent of the phloem of the root and external phloem of the bundles of the hypocotyledonary axis. Its formation in the hypocotyl is always after the formation of external phloem and woody elements to which it is adjoined. In this last observation he is in accord with Scott and Brebner (1891), reporting on *Chironia peduncularis* (p. 277), and other authors, as well as the observations on tobacco reported here. Lamounette also mentions that in those forms in which the internal phloem first appears in the lower portion of the hypocotyl it is usually present in the cotyledons; and conversely, when it first appears at the summit of the hypocotyl, it is usually absent from the cotyledons. He interprets these types as grading insensibly one into another in the different families which possess internal phloem. He has further made two artificial classes: (1) a group in which the hypocotyl shows internal phloem at the time of greening of the cotyledons, such as *Cucurbita maxima* and *Solanum nigrum;* (2) those in which the hypocotyl is deprived of internal phloem at this stage, as *Ipomoea leucantha, Oenothera biennis*, and *Fuchsia corymbiflora*, indicating later relative maturity of the tissue in question. In the stem the internal phloem may differentiate at the same time that other vascular elements appear (Cucurbitaceae), or much later ·(Basellaceae).

From a phylogenetic point of view, Lamounette concluded that the internal phloem must have formed primitively in the stem and secondarily in certain axial hypocotyls, its development being an abnormal formation due to the division of pith cells.

Except for the conclusion that internal phloem arises in the pith and, therefore, ultimately from the ground meristem, the observations recorded here are in complete agreement with those of Lamounette. There is little doubt that for the most part in tobacco internal phloem strands are merely inwardly differentiating branches of external phloem, which would mean, classically, at least, that they originate in the provascular meristem rather than

from the ground meristem, as Lamounette holds. It is becoming increasingly clear, however, that we shall have to revise still further our rather traditionally fixed notions concerning the origin of tissues. The internal phloem in the tobacco seedling appears only at the top of the hypocotyl, which would make it fall into the second of Lamounette's earlier categories. This would likewise call for its being absent in the cotyledons, and it is absent. It would also fall into the second of his artificial classes with regard to time of appearance, for it does not appear until several days after the cotyledons first become green. As pointed out above, it does not differentiate until long after the first-formed xylem and external phloem are mature.

Scott and Brebner (p. 267) describe the transition in the hypocotyl of *Browallia viscosa* H. B. and Kth., which appears to resemble closely the condition in tobacco, as follows:

> On tracing the hypocotyl downwards to the taproot, the changes which we find in the position of the tissues are as follows: the pith gradually thins out; the two lateral bundles disappear, becoming confluent with those of the cotyledons. The primary xylem groups of each cotyledonary pair approach each other and ultimately unite, turning their protoxylem outwards. In the transitional region the strands of internal phloem successively pass out between the converging xylem bundles and one by one reach the strands of the external phloem, with which they fuse. The external phloem strands concentrate themselves on the two sides of the vascular cylinder, between the two centripetal xylem groups, which now represent the cotyledonary pairs. Finally these two groups themselves unite at the center of the root, forming the diarch xylem plate, and at this point the last of the internal phloem strands passes out and joins the normal phloem.

In the only work found on the anatomy of the young tobacco plant, de Toni and Paoletti (1891) discuss the origin of tissues in the root of *Nicotiana* and give a brief account of the structure of root, stem, leaf, fruit, and seed. They agree with Lamounette and Herail with regard to the origin of internal phloem. Lee (1912), however, in a study of seedlings representative of numerous families and genera, finds the internal phloem continuous with the external, except as noted above. In the case of *Nicotiana alata* Link and Otto, he reports that internal phloem is not present, though probably because the seedlings examined were not sufficiently mature.

Artschwager (1918) in studies on *Solanum tuberosum* found strands of external phloem becoming oriented as internal phloem in the hypocotyl, comparable to the findings of Gérard, Scott, Scott and Brebner, and others. Thiel (1931), working with *Solanum melongena*, showed the root-stem transition beginning low in the hypocotyl, the final transition taking place in the cotyledons. His figures show an inward differentiation of external phloem strands until they are finally clearly oriented as internal phloem. At the same time this is taking place, metaxylem (diarch root) is "breaking up" in the hypocotyl into several groups which become reoriented.

Kennedy and Crafts (1931), reporting on the transition region of the older material of root and rhizome of wild morning-glory (*Convolvulus*

arvensis), note that the transition occurs just below the surface of the ground, the xylem being dissected into four or five divisions, and the " internal phloem of the stem divides and passes out on each side of the wedge-shaped xylem segments, ultimately joining the outer phloem of the root." While these results are contrary to the findings of several earlier authors working on younger material in the same family, they agree closely with the observations of Scott and Brebner and others, and with observations on tobacco reported here. It has been shown in the latter that the inward differentiation of branches from the external phloem may take place through cotyledonary gaps, or later through vascular rays. As secondary thickening takes place, the connecting phloem strands are necessarily secondary, being laid down by the cambium along with ray parenchyma. This is in agreement with Scott and Brebner (p. 269) when they suggest that the phloem connecting internal and external groups must come from the cambium as secondary thickening takes place.

Lee (p. 745) states: " Broadly speaking, in the smaller seedlings the transition region is short, and the rearrangements are concluded in the upper part of the hypocotyl; while in the larger seedlings the region of transition is very extended." With regard to primary xylem and size of seedlings, Hill and deFraine (1913, p. 269) conclude that diarchy is characteristic of smaller ones, as is a single median strand at the base of the cotyledonary petiole (p. 268). These generalizations apply rather well to the tobacco seedling.

SUMMARY

1. In the seed the inner walls of the epidermis are heavily thickened, while the outer walls are thin with a light cuticle. The outer walls bend inward at maturity, giving the seed its reticulated appearance. There are three subepidermal layers of thin-walled parenchyma outside a single persisting layer of nucellar tissue. The endosperm consists of three to five layers of thick-walled cells, rich in aleurone and oil droplets.

2. Increase in the length of the hypocotyl is due both to cell division and cell enlargement. No new cells are added in a radial direction; so the number of cells in a transverse section of the seedling hypocotyl before secondary thickening is the same as the number present in the dormant embryo.

3. The pith in the mature seedling extends approximately 1.5 mm. below the level of cotyledonary divergence. All structures of the tobacco seedling are greatly reduced; hence xylem transition from the exarch to the endarch condition is difficult to make out. It takes place about 2 mm. below the cotyledons, on the average, being completed within a perpendicular distance of approximately 1 mm.

4. The internal phloem does not differentiate until several days after all other primary tissues. The external phloem gives rise to four main internal phloem strands, the first differentiating inward through a cotyledonary gap about 1.4 mm. below the level of cotyledonary divergence. This is followed

at successively higher levels by three more strands, until the internal phloem is established at a level 0.7 mm. below the cotyledons. Occasionally there are additional strands. While these inwardly differentiating strands do not follow any exact pattern, it is common to find strands " entering " the cotyledonary gaps, one on either side of each cotyledonary trace. As secondary thickening takes place, the connection between internal and external phloem groups is through phloem parenchyma laid down by the cambium along with the ray parenchyma in the vascular rays.

5. Increase in the size of the cotyledons is likewise by cell division and enlargement, all new cells being laid down in a plane parallel to the upper and lower epidermis (except in the development of vascular bundles), so that the mature cotyledons are the same number of cell layers in thickness as the cotyledons of the dormant embryo. Growth of the cotyledons is at first basipetal, expansion of the blade later taking place equally throughout. The Casparian strip identifies the endodermis as the bundle sheath, well up into the cotyledonary petiole. The midvein is clearly endarch collateral throughout its length, with the phloem more or less in two groups through the petiole. The lower epidermis acts as an absorbing layer to take food material from the endosperm and is thus slightly behind the upper epidermis in development.

6. The cuticle throughout the seedling is thin and should offer little mechanical resistance to the attacks of disease-producing organisms.

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A COMPARATIVE HISTOLOGICAL STUDY OF CROWNGALL AND WOUND CALLUS ON APPLE

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INTRODUCTION

Crowngall is a bacterial disease characterized by the formation of knotty, gall-like malformations on the diseased plant. The prominence of the symptoms and the occasional occurrence of severe epidemics have led to extensive studies extending over a period of many years. The pathogenic nature of the disease was first demonstrated by Smith and Townsend (1907). One of the important developments in the study of crowngall was the discovery that non-pathogenic overgrowths, known as callus knots, may occur on piece-root grafted apple trees. This fact has obviously raised the question of distinguishing between the two types of overgrowth. Such gross criteria as the hardness of the overgrowth and the character of its surface were found to be unreliable. This uncertainty led to a detailed anatomical study of the different overgrowths. In view of the order in which overgrowths were studied in this laboratory, the present paper will deal first with non-pathogenic callus.

Riker and Keitt (1926) and Muncie and Suit (1930) have shown that callus knots are commonly associated with weak graft unions. Fisk (1927) made a preliminary study of overgrowths on apple grafts, and Sass (1932) made a histological study of the healing process during the first growing season in piece-root tongue grafts. Sass described the origin and early stages of development of excess callus. He showed that there is a tendency to produce excess callus wherever there is some obstacle to prompt healing, such as a misfit of the members, or where there is dirt or dead tissue on the plane of contact. He suggested that there are histological similarities between callus knots and certain features of crowngall.

The anatomy of crowngall was first studied in detail by Smith and his associates (1912), who found that the gall consists of proliferated tissues of the host. The proliferated mass is at first parenchymatous, but presently differentiation takes place, producing contorted vascular tissues within the gall. Reviews of this phase may be found in papers by Robinson and Walkden (1923) and Hamdi (1930).

In view of the fact that the more critical anatomical studies on crowngall were made on plants other than the apple, the present work was limited to the study of pathogenic and non-pathogenic overgrowths on apple grafts.

The work included anatomical and microchemical studies and a reëxamination of the question of the distribution of bacteria in the tissue.

MATERIALS AND METHODS

Some of our material was obtained from preserved and imbedded tissues and from grafted trees made for a previous study (Sass, 1932), but most of the material was from new piece-root grafts of Wealthy scion on French crab roots. The cuttings were sterilized in a 1:500 solution of mercuric chloride for five minutes. The grafts were wrapped with waxed twine and stored in sterilized peat until used. The older knots (three to six months old) were taken from grafted trees which had been growing in the field. Crowngall tissue was taken from galls which had developed on inoculated one- and two-year-old grafts. The writers are indebted to Dr. Ross F. Suit for cultures of *Pseudomonas tumefaciens* and for some of the large galls. Only those galls from which Dr. Suit recovered the pathogen were used in these anatomical studies.

Pieces of tissue from crowngalls and callus knots were killed in acetic-formalin-alcohol (alcohol, 50 cc.; water, 40 cc.; U. S. P. formalin, 7 cc.; glacial acetic acid, 3 cc.). The younger stages were imbedded in celloidin. For the histological studies Mayer's hemalum and safranin were used. The tougher tissues in paraffin were cut successfully after hot water treatment.

In staining some of the crowngall material, the particular objective was to differentiate the bacteria from the host tissue. No reference was found in the literature to a method of securing a good differential staining of the crowngall organism on apple. Several stains were tried, including acid hemalum, Mayer's hemalum, iron alum-haematoxylin, safranin-fast green, thionin-orange G, and carbol fuchsin-light green. Mayer's hemalum gave the best results. Smears of the crowngall organism and sections of the gall gave similar staining results. Hemalum stains both the tissue and the bacteria a deep blue; since, however, many of the bacteria occur in open areas, the bacteria are clearly visible. By careful focusing, bacteria that are appressed to the cell wall can be distinguished. Capsuled bacteria are especially well demonstrated by this stain. Thionin-orange G has some value as a stain, although thionin does not stain the individual bacteria very well. This combination stains those regions affected by bacteria vivid green, which contrasts with the orange of the other tissue; this useful indicator demonstrates the location of the bacteria.

Because of the affinity of celloidin for the stain, sections imbedded in celloidin were unsatisfactory for staining bacteria. When such material was destained, the stain came out of the bacteria more rapidly than out of the celloidin.

HISTOLOGICAL STUDIES

The origin of callus and the early stages of internal differentiation. In grafts of Wealthy apple scion on French crab root, the region which may

produce callus includes the primary cortex, the phloem, the cambium, and the indefinite region corresponding to the endodermis and pericycle. The cells on or near the injured surface divide in three planes, producing new tissues consisting of spongy, loosely arranged, undifferentiated cells. This undifferentiated condition of the newly formed cells persists for only a few days. These observations are a repetition and verification of Sass's (1932) results, whose study did not extend much beyond this point in the development of callus.

Differentiation within the callus first appears in localized areas in the form of irregular bands, whorls, cylinders, or spheres (fig. 1–4). The cells in these areas are elongated, stain deeply, and have thin walls, large nuclei, and tapering ends. In these irregular meristematic areas of cambium-like cells, division occurs primarily in one plane, producing layers of derivatives similar to the products of normal cambial activity. By this process the whorls and layers within the knot increase in volume (fig. 1–4); this increase contributes to the increase in the size of the knot.

In the cambiform meristematic areas described above, the derivatives toward the periphery retain meristematic characteristics. The cells produced centripetally elongate and increase in diameter. The walls become thick, lignified, and have full-bordered pits. In some of these cells the thick end wall persists as in tracheids, but in many the end wall is lacking, as in tracheal tubes. As the result of the irregular shape of these elements, the entire "islands" (bands or whorls of tissue) resemble contorted xylem elements (fig. 2, 3, 4). Continued meristematic activity on the periphery of the islands adds irregular layers to the first-formed xylem (text fig. 3; fig. 1, 2, pl. 13).

These meristematic areas produce not only xylem cells centripetally, but also parenchymatous cells centrifugally. The latter cells are not recognizable as phloem elements (text fig. 2, 3; fig. 1, pl. 13), but resemble cortical parenchyma. This centrifugal formation of spongy tissue further increases the bulk of the knot. The increase in the size of a callus knot is evidently the result of two types of meristematic activity operating simultaneously: proliferation of the surface cells of the knot, and meristematic activity of localized areas within the knot.

Duration of meristematic activity in callus knots. The duration of meristematic activity in callus seems to be determined by external conditions such as moisture and temperature. Considerable variation was observed in the same lot of grafts; on some grafts a periderm was formed on the surface of the callus in ten days (fig. 3, pl. 13); on other grafts continued meristematic activity on the surface prevented the periderm formation for two or three months. Moreover, the tissues just beneath the periderm of the callus may become reactivated. Two-year-old knots were found to have surface layers or localized masses of actively dividing tissues. Microscopic examination of such knots showed that the reactivated tissue had burst through the protective inert surface layer of periderm.

The localized internal meristematic areas can be recognized in stained sections, and active cell division in these areas is almost invariably evident two to three weeks after grafting. The duration of cell division in any given area is obviously difficult to estimate, for it is necessary to kill the tissue for examination. However, in callus knots three weeks old, internal areas were found that had already attained maximum differentiation into xylem and parenchymatous elements, and were without the typical meristematic layer (fig. 4). Evidently, a given internal meristem may carry on cell division for three weeks or possibly less. New meristematic whorls or bands subse-
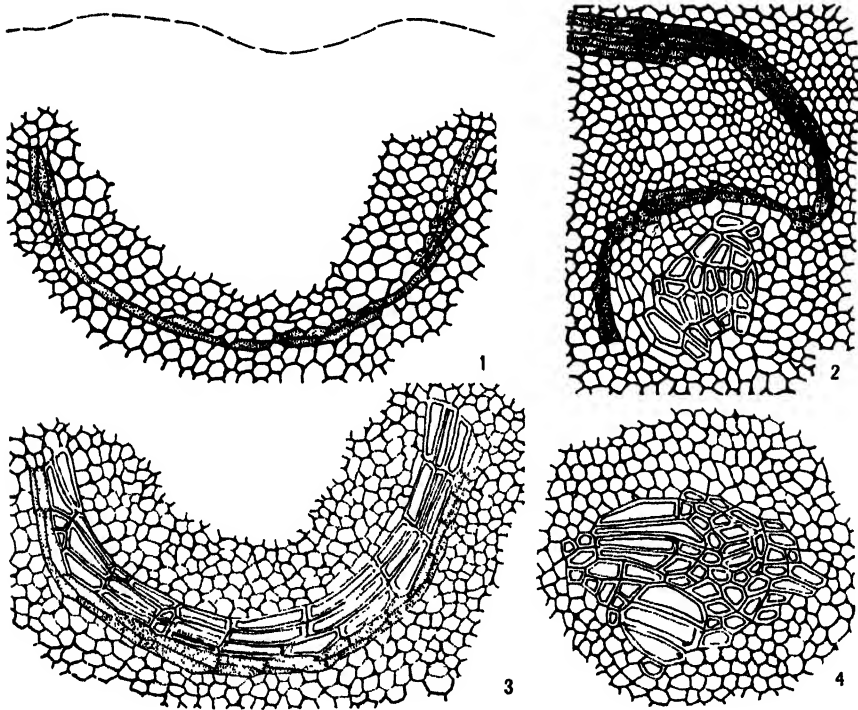
Fig. 1-4. Callus. Fig. 1. First evidence of the meristematic layer of cambium-like cells in three-weeks-old callus. ✕ 40. Fig. 2. Internal meristematic area in six-weeks-old callus. ✕ 40. Fig. 3. Further development of cells as seen in figure 1. ✕ 40. Fig. 4. Mature xylem area in three-weeks-old callus. No further meristematic activity is probable in this region. ✕ 40.

quently arise farther out in the parenchymatous peripheral zone of the callus (text fig. 2; fig. 1, pl. 13).

Continuity of xylem elements of callus knots. The food and water necessary for the growth of the callus come from the tree, presumably through some kind of vascular system. The only vascular elements in the callus knots are the products of the activity of the internal meristematic regions pre-viously described. We have demonstrated that in sections these elements

do not have the appearance of normal vascular tissues. There is no typical phloem and only a highly contorted form of xylem. Serial sections indicate that these xylem masses are discontinuous, and range from 30 to 600 μ in greatest dimension. Callus knots were macerated with potassium chlorate and hydrochloric acid, and the disintegrated material was examined. In the macerated tissues, small cylinders, irregularly ovoid, and spherical masses of hard, xylem-like tissues were found. With less vigorous maceration as many as two or three globose xylem masses were observed to be loosely connected by relatively large amounts of parenchymatous tissue. By continued vigorous maceration such xylem masses can be separated. Thus the serial sections and macerations indicate that there is no effective vascular continuity between the numerous xylem masses within the knot and between these specialized xylem masses and the vascular system of the tree.

Callus knots having a diameter of 1 cm. or more have a more or less solid woody core, and this core is apparently continuous with the wood of the growing tree. From microscopic examination of these cores, as well as from the study of the development of the woody " islands " in the knot, it is evident that the woody core of the knot is formed by the virtual coalescence of many separate " islands." The apparent continuity of the core of the knot with the xylem of the tree is the result of the enveloping of the base of the core by the cambial activity of the tree.

THE STRUCTURE OF APPLE CROWNGALL

The initiation and early stages of apple crowngall were not studied in the present work. From the extensive literature on the structure of crowngall in a number of other plants, it is safe to infer that in the apple, crowngall consists of proliferated tissues of the "bark."

From the study of the peripheral regions of small galls (3–6 months old), it is clear that the growth of the gall is due in part to surface proliferation. Proliferation on the surface continues intermittently, probably depending on moisture conditions. Small masses of snow-white new tissue were found on the cracked surface of two-year-old galls.

Internal meristems appear in the form of whorls, layers, cylinders, and spheres of deeply staining cells. The cambium-like activity of these areas closely parallels the process observed in callus knots. This activity contributes to the enlargement of the crowngall. Simultaneously with the appearance of new meristematic zones near the periphery of the gall, the older, formerly meristematic areas deeper in the gall undergo differentiation into contorted xylem elements. Some of the cells elongate and develop thick, well lignified walls pierced by full-bordered pits. These cells vary from almost straight to crescent shape. The end walls may be either transverse or tapered, but tend to persist as in tracheids. In some instances the end walls become porous as in vessels. In a given area the maturing of cells proceeds centrifugally; lignified cells in the center merge into an indefinite meris-

tematic area, the outer derivatives of which merge into the parenchyma of the surface. In apple crowngall, occasional internal areas, very suggestive of the dicotyledonous bundle, are found. These areas have fairly well-defined xylem, containing parenchymatous strips resembling vascular rays (fig. 2). Certain isolated groups of cells resemble tangential views of vascular rays. Among these cells are interwoven long, thin-walled cells resembling fusiform, septate cambium initials (fig. 7). In addition to the specialized tissues mentioned above, the ground parenchyma contains isolated or clustered fibers. These are typical sclerenchyma cells (fig. 8). The above observations indicate that certain features of crowngall tissue resemble the tissues of the normal stem.

Near the periphery of the gall a distinct zone of cells, one to several layers in thickness, is found. These cells are polygonal, isodiametric, and take a deep stain (fig. 4). The dark color of these surface cells is due in part to inclusions which are found as black granules or rod-like bodies scattered throughout the cell. In older galls these bodies are often so numerous and tightly packed within the cell that the whole mass is black. It appears that these dark cells in crowngall are particularly numerous in the region of the "bacterial pockets" to be described later. In some galls, however, even large ones, such blackened cells are uncommon or absent. In that case the gall may be limited by an indefinite surface layer of closely packed brownish cells resembling the periderm found in wound callus. The presence of the peculiar layer of dark polygonal cells seems to be a fairly good diagnostic character of crowngall.

The growth of the localized internal areas (fig. 1), as well as irregular surface proliferation, produces the rough, wrinkled surface of the gall. Growth of the parenchymatous area surrounding the fissures on the surface tends to bring the sides of the fissure together (fig. 10, 12, 14). The two margins may ultimately close in. Such enclosures are always bordered by cells containing characteristic deposition of gummy materials. These areas, not previously reported except in a vague sense by Toumey (1900) may be correctly termed "bacterial pockets." The occurrence of bacteria in these pockets will be described presently.

MICROCHEMICAL TESTS

Fresh callus was obtained by storing newly cut scion wood in sterilized peat; galls were obtained from young apple trees which had been inoculated with the crowngall organism. Four-weeks-old wound callus and three-months-old crowngalls were used. At the end of four weeks wound callus was present in sufficient quantity and of such a typical nature as to furnish material for the tests; due to seasonal conditions, however, the galls did not develop as rapidly and were left growing longer to furnish sufficient material. Isolations made from the galls yielded the pathogen which proved pathogenic upon inoculation into the tomato. In addition to testing fresh tissues, callus

and gall material were preserved directly in 70 per cent alcohol and then subjected to the microchemical tests described in table 1.

The method of killing and storing material in 70 per cent alcohol makes it possible to collect tissues at a remote nursery and bring them to the laboratory for critical examination. The results obtained with fresh tissue and preserved material were identical.

The tissues of callus and gall give practically identical, positive tests for cellulose, suberin, pectin, and lignin. The pectin reaction is slightly weaker in gall than in callus.

The tannin test has some diagnostic value, being negative in callus and positive in gall. In the particular test used for gums, the occurrence of which was believed responsible for the dark color of certain cells of crowngall, callus tissue gave a negative test, while gall tissue gave only a slight or negative test. The value of this test as a specific indication for such complex and diverse substances as the " gums " may well be questioned.

TABLE 1.   *Microchemical tests used in the comparative study of the tissues of callus and crowngall (after Molisch, 1913)*

Test for:	Reagents	Reaction
Cellulose	.3 g. I 1.5 g. KI $\Big\} + H_2SO_4$ 100 cc. c.p. water	Blue cell wall if cellulose is present; suberin a pale yellow.
Suberin	.5 g. Sudan III in 100 cc. 70% alcohol	Suberin a golden yellow, other cells colorless.
Pectin	1 part ruthenium red in 1000 cc. water (c.p.)	Pectic substances a light pink to light red in color.
Lignin	1 g. phloroglucin in $\Big\} + 25\%$ HCl 10 cc. 95% alcohol	Lignin stains red to violet.
Tannin	10 g. ferric chloride in 100 cc. water (c.p.)	Tannins color blue to green.
Gums	4 g. orcein in 100 cc. water (c.p.) $\Big\} +$ HCl	Gums color purple to blue.

OCCURRENCE OF BACTERIA IN CROWNGALL TISSUE

Bacteria on the surface of the gall. Sections of apple crowngall showed the presence of bacteria on the exterior of the gall, occurring either in the active or capsuled condition (fig. 11, pl. 14). Although innumerable bacteria are undoubtedly removed during the necessary washing and the imbedding process, our preparations show that as a rule bacteria are much more numerous on the outside of the gall than on the inside. Bacteria are especially evident in the long fissures on the convoluted surface of the gall (fig. 9, pl. 14; text fig. 5), but we can safely assume that bacteria are present in large numbers

upon the entire surface of the gall.   This seems to agree with the findings of Hamdi (1930), Robinson and Walkden (1923), Colley (1931), and Smith and Townsend (1907), who point out that *Pseudomonas tumefaciens*, being a distinct aërobe, grows only in such areas as have access to abundant oxygen.

Closely bordering the surface of the gall there are usually extensive areas of cells which are not as yet entirely broken down (fig. 12, pl. 14; text fig. 5). They may be in fairly good condition except for plasmolysis and a general darkened condition.   Capsuled and active bacteria can be found in large numbers in such areas.   As the outer portions of the gall are being con-
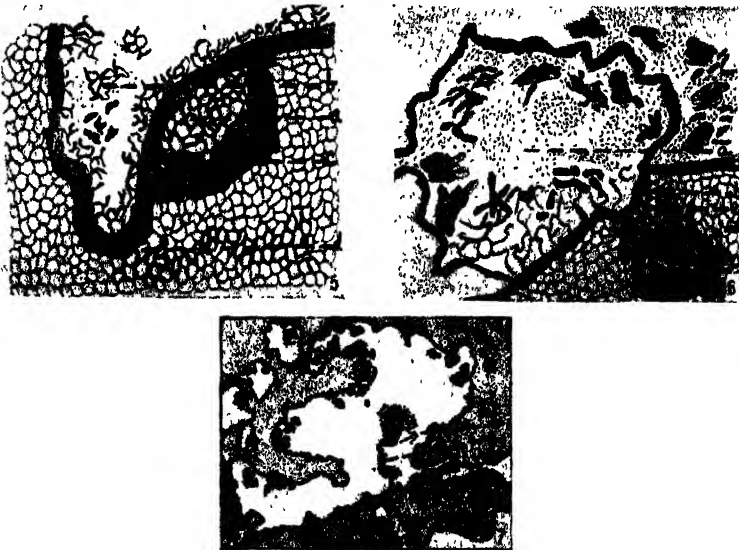
Fig. 5–7.   Crowngall.   Fig. 5.   Surface of six-months-old gall.   Blackened area at *a* precedes general breakdown such as pictured at *b*.   Note pseudoperiderm at *c* and beginning of cell blackening at definite region *d*.   × 50.   Fig. 6.   Section of six-months-old gall, showing completely broken down bacterial pocket containing many bacteria at *a*, protective pseudoperiderm at *b*, and blackened area of cells extending inward from the point *c*.   The bacterial pocket at *d* shows a fissure in which bacteria may often be seen. × 50.   Fig. 7.   Interpretative drawing of region shown in figure 15 of plate 14.   Note the broken down condition of the cells forming a schizogenous cavity, showing capsuled and active bacteria at *a* and *b*, respectively.   × 110.

tinually sloughed off, active and capsuled bacteria are carried out into the broken-down cell areas and finally into the soil, where the tissue disintegrates. Thus, we see that there is a constant supply of viable bacteria thrown off into the soil, easily accounting for the fact that soil is very heavily infested even after only one crop of diseased plants.

Bacteria within the gall.   In addition to the bacteria which live for the most part on the surface of the gall, there are bacteria in the interior of the gall.   Their occurrence will be described under three heads—namely, (1)

bacteria inside the cells, (2) bacteria in the "bacterial pockets," and (3) bacteria in the broken-down schizogenous cavities.

In the young crowngall tissue bacteria are present in large numbers in the cells of the outer tissues. Here they are scattered throughout the protoplasm which is in early stages of senility. They can be distinguished from other cell inclusions in this stage. Cell inclusions are generally of a granular, irregular nature; the bacteria are somewhat regular in outline, are rod-shaped, and are rather bluntly rounded at the ends. Mitochondrial bodies, even though rod-like, are not as regular in shape and are not stained as characteristically by the methods employed. The shape of the bacteria in these cells near the surface is identical with that of those found on the surface of the gall (fig. 13, pl. 14). The cells which thus harbor the bacteria are found entirely in the surface layers, again substantiating the claim that the bacteria must have a good supply of oxygen. A little deeper in the gall there is a dark-stained, gum-filled layer which has been described as being characteristic of crowngall (fig. 13, pl. 14). Although there are bacteria occurring within the outer cells of the gall, these cells are soon " walled off " by the dark layer, and subsequently are sloughed off into the soil.

Bacteria occurring in the "pockets" formed by the closing in of the adjacent walls of the convoluted surface of the gall are usually in the capsuled condition and seem to be present in small numbers. Frequently one observes a " pocket " surrounded by apparently normal host cells (fig. 14, pl. 14), which have a clear blue color when stained with Mayer's hemalum. Occasionally there seems to be a rupture of the enclosed cells, and a few capsuled bacteria, tightly adhering to the broken cell walls, can be distinguished.

Figure 15 is an enlargement of region d in text figure 6. The area surrounding such a pocket becomes blackened and broken down completely. This blackening starts at a definite point at one of these " bacterial pockets " and proceeds until the whole area is disorganized.

In the disorganizing outer tissues of older galls there are large schizogenous cavities (fig. 16, pl. 14) which contain many bacteria, usually capsuled. Bacteria are found relatively deeper in the gall when the layer of dark polygonal cells (fig. 9, pl. 14) is absent than when this " protective " layer is present. Even here the bacteria do not occur very far into the hyperplastic tissue.

DISCUSSION

The principal objective of the present study was to find diagnostic differences between non-pathogenic callus knots and crowngall on the apple. The results have served to emphasize the similarities, at least during the early stages of overgrowth formation. Since both types of overgrowth arise by proliferation of the same tissues of the apple tree, similarities were to be expected. The possibility of subsequent development into structurally different overgrowths was entertained, but a comparative study of various stages of development has shown a similar course of differentiation in both callus

and crowngall. Some structural characteristics of doubtful diagnostic value were observed.

In view of Sass's (1932) report that graft callus may be a mixture of stem and root callus, the varied character of the surface layer on callus knots was not surprising. However, many of our preparations of callus showed a periderm identical with that of the normal apple stem. Crowngall does not have this type of periderm. The outer layers of crowngall may have the appearance of being distinctly the products of disorganization. On the other hand, in actively growing galls the surface may be parenchymatous and quite indistinguishable from growing callus.

Internal differentiation in graft callus and crowngall is strikingly similar. The contorted strands and islands of distorted xylem elements, occasionally organized to resemble a dicotyledonous bundle, are common to both types of overgrowth. Sclerenchyma cells seem to occur only in crowngall, but the presence of small masses of xylem having greatly reduced pitting makes this diagnosis uncertain.

The presence of xylem elements in overgrowths raises the question of vascular continuity between the overgrowth and the tree. A large graft callus knot or a large crowngall has a woody core that is attached to the xylem cylinder of the tree. In both crowngall and wound callus the woody core is produced by the coalescence of numerous strands or islands of lignified tissue. These islands were shown to arise as meristematic areas which enlarge and differentiate centrifugally until extensive coalescence occurs. Simultaneously with the formation of the overgrowth the cambium of the tree is active, and the progressively differentiating xylem of the tree comes into contact with the woody core of the overgrowth. Because of the mode of origin of the woody core of the overgrowth and the contorted nature of the xylem elements of the core, the path of materials between the tree and the overgrowth is highly tortuous. There may, indeed, be some objection to regarding the core of the overgrowth as a vascular system.

In view of the histological evidences of similarity between callus and crowngall, microchemical comparisons may be expected to show similar reactions, in so far as the cell walls of the various tissues are concerned. The tests show such similarity, except for the presence of tannin in crowngall and not in callus. Chemical differences may be ascribed either to reactions of crowngall tissue to the organism or to products of the metabolism of the bacteria. The microchemical tests used here are more likely to detect changes in apple tissue than to detect bacterial products. It would probably be profitable to make a comprehensive and detailed study of the metabolism of *Ps. tumefaciens,* especially its by-products, and then to make microchemical tests on the respective tissues of graft callus and crowngall. Concerning the distribution of bacteria in crowngall tissue, the histological evidence supports the experience of pathologists, who find that isolations are more readily obtained from superficial tissues. The very simple staining

technique used in this work stains satisfactorily the bacteria in a smear, on the surface of a gall, or in the schizogenous cavities near the surface. Failure to demonstrate bacteria in the deeper tissues may be ascribed either to the absence of bacteria or to a change in the size or staining reaction of bacteria that may be present. This study has given no clue to the true situation.

It is now generally believed that the crowngall organism does not occur in living cells. The present study supports this view. The apparently normal cells shown in figure 13 are very near to the disorganized surface area and are probably dead. This may allow the bacteria to enter and distribute themselves throughout the dying protoplast. Such cells are in a short time " walled off " from the rest of the proliferating host tissue. A more reliable criterion of the condition of the cells that harbor the bacteria is desirable.

SUMMARY

1. Crowngall and non-pathogenic callus knots on apple grafts were compared with respect to histological development and the microchemical reactions of their respective tissues. The distribution of bacteria in crowngall was studied.

2. Both types of overgrowths have the following features in common:

The overgrowth consists of proliferated host tissue, derived from tissues external to the xylem cylinder. Within the parenchymatous overgrowth internal differentiation takes place in the form of isolated spheres, whorls, cylinders, or sheets of meristematic cells.

In these " meristematic islands " the peripheral cells divide in a regular, cambium-like manner, producing stratified derivatives on the inside and parenchyma on the outside.

The meristematic islands undergo centrifugal differentiation into lignified, contorted xylem elements. Meristematic activity in a given island terminates in about three weeks.

New meristematic islands arise progressively in the parenchymatous margin of the overgrowth.

Enlargement of the overgrowth is partly by proliferation on the surface and partly by meristematic activity in the internal islands.

Microchemical reactions of callus and crowngall are practically identical for cellulose, pectin, lignin, and gums. No true vascular connection was found between the overgrowth and the tree.

The apparent continuity of the woody core of the knot with the main stem is brought about by the gradual coalescence and differentiation of the numerous meristematic " islands."

3. Crowngall and callus-knot differ in the following respects:

Crowngall usually has near the surface a zone of dark, polygonal, close-fitting cells, easily distinguished from the surrounding parenchyma.

Graft-callus usually has a periderm similar to the periderm of the normal stem.

The test for tannin was positive in crowngall tissue and negative in callus tissue.

Sclerenchyma cells are common.

4. *Pseudomonas tumefaciens* was found abundantly on the surface of the galls of the apple, in the schizogenous cavities, and in partially disorganized cells near the surface.

This work has been carried out in connection with the crowngall project, in coöperation with the Crop Protection Institute, the University of Wisconsin, and the United States Department of Agriculture. The authors wish to acknowledge their indebtedness to Dr. J. E. Sass, under whose direction this work was carried out, and to Dr. I. E. Melhus for many valuable suggestions.

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EXPLANATION OF PLATES

PLATE 13

Fig. 1.   Graft callus, three weeks old.   Note surface proliferation *a;* new meristematic strand *b;* older strand with xylem at *c,* and cambiform layer at *d.*   X 40.

Fig. 2.   Contorted arrangement of elements in a six-weeks-old callus.   X 40.

Fig. 3. A callus twelve weeks old. Note the typical periderm such as found on normal stem. Notice the thick walls and the characteristic cell shape. Some of the peridermal cells still contain their nucleus. × 50.

Fig. 4. Periderm of true crowngall, one year old; polygonal cells containing crystalline and gummy materials are characteristic of crowngall. × 50.

Fig. 5. Surface of two-year-old gall. Note the pseudoperiderm, a type found on either crowngall or callus. This may be an early stage of development of either type of limiting layer. × 50.

Fig. 6. Stem-like structure of localized area in true crowngall of two seasons' growth. This does not appear as frequently in the callus. × 40.

Fig. 7. Ray-like groups of cells evident in the true crowngall of one season's growth. × 50.

Fig. 8. Thick-walled, lignified fibers present in true crowngall, one year old. Such fibers do not occur in the wound callus. × 75.

PLATE 14

Fig. 9. Section of three-months-old gall. Note deep fissure at *a* and bacteria at *b*. Note also black, gum-filled layer at *c*, one to several cells in thickness, and d.sintegrated cells at *d*. × 50.

Fig. 10. Exterior surface of six-months-old gall. Note the fissure at *a* being closed in at the surface line by the growth of the gall at *b* and *c*. Note the deposition of gummy materials surrounding the "bacterial pocket." × 50.

Fig. 11. Section of exterior surface of six-months-old gall, showing protective pseudoperiderm layer and numerous bacteria on the relatively smooth outer surface of the gall. × 220.

Fig. 12. Surface of three-months-old gall. Note dark pseudoperiderm at *a*, the former periderm at *b* which has been broken by the expanding of the gall. The group of cells at *c* which are being cut off from the gall are still in fairly good condition, excepting general darkening and plasmolysis. Note meristematic area at *d* where surface proliferation occurs. × 40.

Fig. 13. Section of three-months-old gall. Outer portion of the gall showing the bacteria within the dead cells at *a*. Note also capsuled bacteria at *b* and the beginning of a pseudoperiderm layer at *c* which will ultimately "wall off" the bacterial infected region. × 330.

Fig. 14. Hyperplastic tissue in the process of enclosing "bacterial pockets." Note completely enclosed pockets at *a* and *b*, characterized by the gummy deposition of material. A third pocket at *c* would ultimately be formed by the growth of the gall at *d* and *e*. Bacteria may often be seen in the capsuled condition at *f*. × 40.

Fig. 15. Enlarged view of schizogenous cavity of six-months-old gall, showing· capsuled bacteria at *a* and active bacteria at *b*. For interpretative drawing of this region, see text fig. 7. × 220.

Fig. 16. Surface of three-months-old gall. An area such as pictured in fig. 13 excepting that general breakdown of the cells has occurred, resulting in distintegrated tissue which harbors many bacteria. Note pseudoperiderm at *a*. × 50.
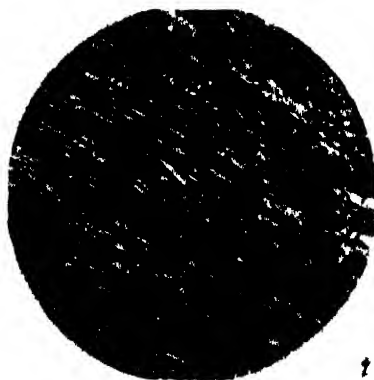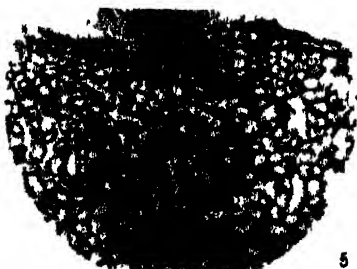
SYLWESTER AND COUNTRYMAN: CROWNGALL AND WOUND CALLUS

NUCLEAR DIVISIONS IN THE TAPETAL CELLS OF
GALTONIA CANDICANS

FRANK H. SMITH

INTRODUCTION

The increasing number of descriptions of nuclear divisions in tapetal cells has established the fact that these divisions are mitotic rather than amitotic, as was previously considered by many to be the case. Strasburger (1882) described typical mitoses of tapetal nuclei. Duggar (1899) also early described mitoses in the tapetal cells of *Bignonia venusta*, which are normally binucleate during the stages of microsporogenesis following synizesis. Tischler (1906) described mitotic division of the nuclei in tapetal cells of *Ribes*. The second nuclear division, however, he found to be amitotic. He also found nuclei with the tetraploid chromosome number and concluded that such nuclei resulted from a fusion of the two diploid nuclei formed after the first division. Winkler (1906) described a fusion of nuclei in the tapetal cells of *Wikstroemia indica*. Mitotic divisions occur in the tapetal cells, resulting in a 2–6 nucleate condition. These nuclei usually fuse to form giant nuclei, but in some cases they remain separate. Tahara (1910) found in *Morus indica* that during the early stages of the heterotypic division the tapetal cells are binucleate. Their nuclei may fuse to form a single tetraploid nucleus which in turn may divide mitotically, or they may divide separately to form four nuclei. The four nuclei then fuse in pairs, so that each cell contains two tetraploid nuclei which may later divide mitotically.

Bonnet (1912) made an extensive study of the tapetal cells in several genera of angiosperms and found mitosis to be the only method of nuclear division in the material examined. In *Yucca* the first tapetal nuclear division occurs before synizesis; in *Fuchsia* it occurs after synizesis. The second divisions are simultaneous in each cell. In some cases two of the spindle poles converge, so that during the telophases two of the four daughter nuclei fuse to form a single tetraploid nucleus. Thus the cell contains one tetraploid and two diploid nuclei. Bonnet also described a fusion of nuclei in a resting condition which results in the formation of tetraploid and octoploid nuclei. Gates and Rees (1921) found only mitotic nuclear divisions in the tapetal cells of *Lactuca*. The first division occurs at the start of synizetic contraction, and by the time of maximum synizetic contraction the tapetal cells are all binucleate. Some of them remain binucleate; others undergo a second nuclear division as the pollen mother cells reach the open spireme stage. The

second divisions are simultaneous throughout a loculus. A three-nucleate condition results from a crowding of the spindles, as Bonnet (1912) found in *Yucca* and *Fuchsia*. During the later stages of the meiotic divisions the nuclei in each tapetal cell of *Lactuca* may fuse. Gates and Rees also describe incomplete synizesis in cells that represent a transition between tapetum and sporogenous tissue. Campin (1925) found in *Nolana* that the first nuclear divisions occur while the pollen mother cells are in the open spireme stage. The tapetal cells are usually binucleate, but some four-nucleate cells are present. Meyer (1925) concluded that in *Leontodon autumnalis* tetra-, hexa-, and octoploid nuclei result from a splitting of the chromosomes and the failure of the nucleus to divide. Usually there are three divisions, after which the tapetal cells are four- or eight-nucleate. He found no nucleoli in these multinucleate cells. Maheshwari (1929) described mitotic nuclear divisions in the tapetal cells of *Boerhaavia*, resulting in a binucleate condition by the time the pollen mother cells have reached the open spireme stage. Homedes (1928), Mascré and Thomas (1930), and Cooper (1931) found in various species of angiosperms that the first divisions of the tapetal nuclei occur while the pollen mother cells are undergoing synizesis. Mascré and Thomas described nuclear fusion after two or four nuclei were formed.

Juel (1915) described the tapetal cells of *Galtonia candicans* as regularly binucleate. He considered the tapetum to be of the secretory type in contrast to the plasmodium type found in *Arisaema*. The diploid chromosome number of *Galtonia* is sixteen, as first determined by Schniewind-Thies (1901). The material used in this study of the tapetal cells was fixed in Flemming's medium solution after treating the anthers for about forty-five seconds in Carnoy's solution (60 cc. of absolute alcohol, 10 cc. of glacial acetic acid, and 30 cc. of chloroform). The usual paraffin method was used and the sections were stained in Heidenhain's iron-alum haematoxylin.

OBSERVATIONS

Nuclear divisions in the tapetal cells of *Galtonia candicans* occur by typical mitosis. Amitotic divisions are not found. The cycle of the chromonemata · in the tapetal nuclei is essentially the same as in the somatic cells (Smith, 1932). The relation of the satellite chromosomes to the nucleolus already described for somatic nuclei (Smith, 1933) also holds here, with the exception that in tetra- and octoploid tapetal nuclei two and four pairs of satellite chromosomes are found instead of one pair as in diploid cells.

When the pollen mother cells are in a resting condition, the anther wall consists of four layers of cells—epidermis, endothecium, a single transition layer, and an innermost layer next to the sporogenous tissue. Some of the cells of this inner layer do not divide but are transformed directly into tapetal cells. Others undergo tangential division (fig. 5, pl. 15). The inner cells resulting from these divisions become tapetal cells and the outer ones add a second layer to the transition region, which may thus be either one or two cells

in thickness. Some radial divisions also occur in the tapetal layer at this time, both daughter cells becoming tapetal cells. Frequently the last premeiotic divisions in the sporogenous tissue occur about the time of these cell divisions in the inner layer of the anther wall.

Usually the first nuclear divisions in the tapetal cells after the completion of the cell divisions just described occur as the nuclei of the pollen mother cells are beginning to undergo the synizetic contraction. Not infrequently, however, these tapetal nuclear divisions are delayed until the time of maximum synizetic contraction (fig. 6, pl. 15). At or shortly after this period of maximum contraction, therefore, most or all of the tapetal cells are binucleate.

At the time of the open spireme stages in the microspore mother cells the two diploid nuclei in each tapetal cell undergo a second mitotic division. These divisions are simultaneous in each cell, but not throughout the loculus. The results of these divisions vary considerably. In no cases were nuclear fusions observed; that is, no nuclei were fusing while in a resting condition.

Fig. 1-4. Semi-diagrammatic drawings of second nuclear divisions in tapetal cells Fig. 1. Late prophase stages, the two nuclear cavities coalescing as the nuclear membranes disappear. Fig. 2. The two nuclei have formed separate spindles which converge at one end. (See fig. 9, pl. 15). Fig. 3. Equatorial-plate stages with the two spindles lying end to end in the same plane. (See fig. 11, pl. 15.) Fig. 4. Late anaphase stages with the two spindles approximately at right angles to each other. All figures × 1750.

Most frequently the two nuclei are found close together as they are passing through the prophases. Then, during the late prophases, as the nuclear membranes disappear, the two nuclear cavities coalesce so that both diploid sets of chromosomes are free in the same cavity (text fig. 1; fig. 7, pl. 15). Thus the nuclei have not fused in the true sense of the term, yet the tetraploid number of chromosomes is present in a single cavity. All thirty-two chromo-

somes move to a single equatorial plate (fig. 10, pl. 15). As the daughter chromosomes separate, thirty-two pass to each pole of the spindle (fig. 13, pl. 15). The result is the formation of two tetraploid nuclei in each cell (fig. 15, pl. 15). The transition layer shows signs of degeneration at this time (fig. 7, 8, 11, pl. 15).

In many cases, however, the two diploid nuclei do not coalesce during the late prophases, but remain separate and form two distinct spindles. If the two spindles are end to end in approximately the same plane, two of the spindle poles coincide (text fig. 3; fig. 11, pl. 15). The two anaphase groups of chromosomes at these poles are thus very close together (fig. 8, pl. 15), and during the telophases both groups of chromosomes are incorporated into a single large nucleus (fig. 14, pl. 15). The result is a three-nucleate condition (fig. 12, pl. 15), one nucleus being tetraploid and two diploid. The same result is obtained if the two spindles converge at one side of the cell (text fig. 2; fig. 9, pl. 15). If the two spindles are at right angles to each other (text fig. 4), or in any other position so that two poles of different spindles do not coincide, then four diploid nuclei are formed (fig. 15, pl. 15). This occurs rather infrequently as compared with the conditions which result in the three-nucleate tapetal cells. As a rule the number of chromosomes that are incorporated in a nucleus can be estimated by the size of the nucleus. However, because of the crowded conditions in the tapetal cells, there is no constant relation between the chromatin content of a nucleus and its size.

No instances were found in which four diploid nuclei were undergoing a third division. Only one cell was observed in which three nuclei were dividing. These divisions were not exactly simultaneous, but two of the nuclei were in the late prophases, while the third had reached the equatorial-plate stage (fig. 16, pl. 15). The pollen mother cells in the adjoining loculus were in a late open spireme stage at this time. Cells containing two tetraploid nuclei show rather frequent division figures, all of which are found while the adjacent pollen mother cells are in diakinesis. The two tetraploid nuclei may behave in any of the ways described for the two diploid nuclei resulting from the first nuclear division. If they coalesce during the late prophases, sixty-four chromosomes move on to a single plate, and a like number of daughter chromosomes move to opposite poles of the spindle in a perfectly regular manner (fig. 19, pl. 15). In consequence the cell contains two octoploid nuclei.

If the spindles of the two tetraploid nuclei are formed separately in such a position that two of their poles coincide (fig. 17, pl. 15), then three nuclei are formed, of which two are tetraploid and one octoploid. If the two spindles are approximately at right angles to each other (fig. 18, pl. 15), four tetraploid nuclei are formed. The only difference noted in these (third) divisions as compared with the previous ones is that the chromosomes appear somewhat more slender. After the nuclei of the pollen mother cells have passed through diakinesis, no nuclear divisions are found in the tapetal cells.

By the time of the homeotypic division in the microspore mother cells the tapetal cells have enlarged greatly and contain large vacuoles. Their nuclei begin to show signs of degeneration at this time. Chromatic material coagulates on the nuclear network, and the nuclei may fuse, so that one or two nuclei are found in a tapetal cell at this time (fig. 20, pl. 15). These are the only true nuclear fusions observed, and here they are evidently the result of degeneration. As the microspores are formed, the tapetal cells become densely granular and increasingly chromatic, and their large vacuoles disappear (fig. 21, pl. 15). The tapetal nuclei themselves appear vacuolate, with dense patches of coagulated material. The transition layer has disappeared entirely, and the endothecium is sharply differentiated from the epidermis. While the tapetum in *Galtonia* is of the secretory type as described by Juel (1915), when the pollen grains are formed, remnants of the tapetal cells break loose from the endothecium and float out among the pollen grains (fig. 22, pl. 15).

SUMMARY

1. All nuclear divisions in the tapetal cells are typically mitotic.

2. The first division of the tapetal nucleus occurs as the pollen mother cells are undergoing synizetic contraction.

3. The second nuclear division in the tapetal cells occurs while the pollen mother cells are in the open spireme stages.

4. A third division may occur while the pollen mother cells are in diakinesis.

5. Tetraploid and octoploid nuclei result from the second and third divisions, respectively, when the two nuclear cavities coalesce during the late prophases as the nuclear membranes disappear.

6. One tetraploid and two diploid nuclei, and one octoploid and two tetraploid nuclei, may be formed after the second and third divisions, respectively, if two spindle poles coincide so that two anaphase groups of chromosomes from different spindles are incorporated into a single nucleus.

7. After the second division four diploid nuclei may be formed. These were not observed to undergo a third division.

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EXPLANATION OF PLATE 15

All photographs except figures 9, 14, 16, and 22 were taken with a Spencer 4-mm. objective and a Zeiss 12 × compensation ocular. For figures 9, 14, and 16 an 18 × ocular was used. For figure 22 a 16-mm. objective and a 12 × ocular were used.

Fig. 5. A young anther, showing four layers in the anther wall at the time the pollen mother cells are in a resting condition. From the right the layers are epidermis, endothecium, transition layer, and an innermost layer. Divisions are occurring in this inner layer which will add to the transition layer and give rise to the tapetum. × 500.

Fig. 6. Time of maximum synizesis. The tapetal cells are binucleate, or the nucleus in each is undergoing the first division. × 500.

Fig. 7. The coalescence of the nuclear cavities during the late prophases of a second division. (See text fig. 1.) × 500.

Fig. 8. Anaphases of a second division with the spindles end to end approximately in the same plane. Two of the groups of daughter chromosomes are thus side by side. The transition layer is beginning to degenerate. × 500.

Fig. 9. Metaphases of a second division with two spindle poles converging above. (See text fig. 2.) × 750.

Fig. 10. Equatorial plate of a second division with thirty-two chromosomes on the plate. This follows a nuclear fusion like that shown in figure 7. The cell below contains two diploid nuclei. × 500.
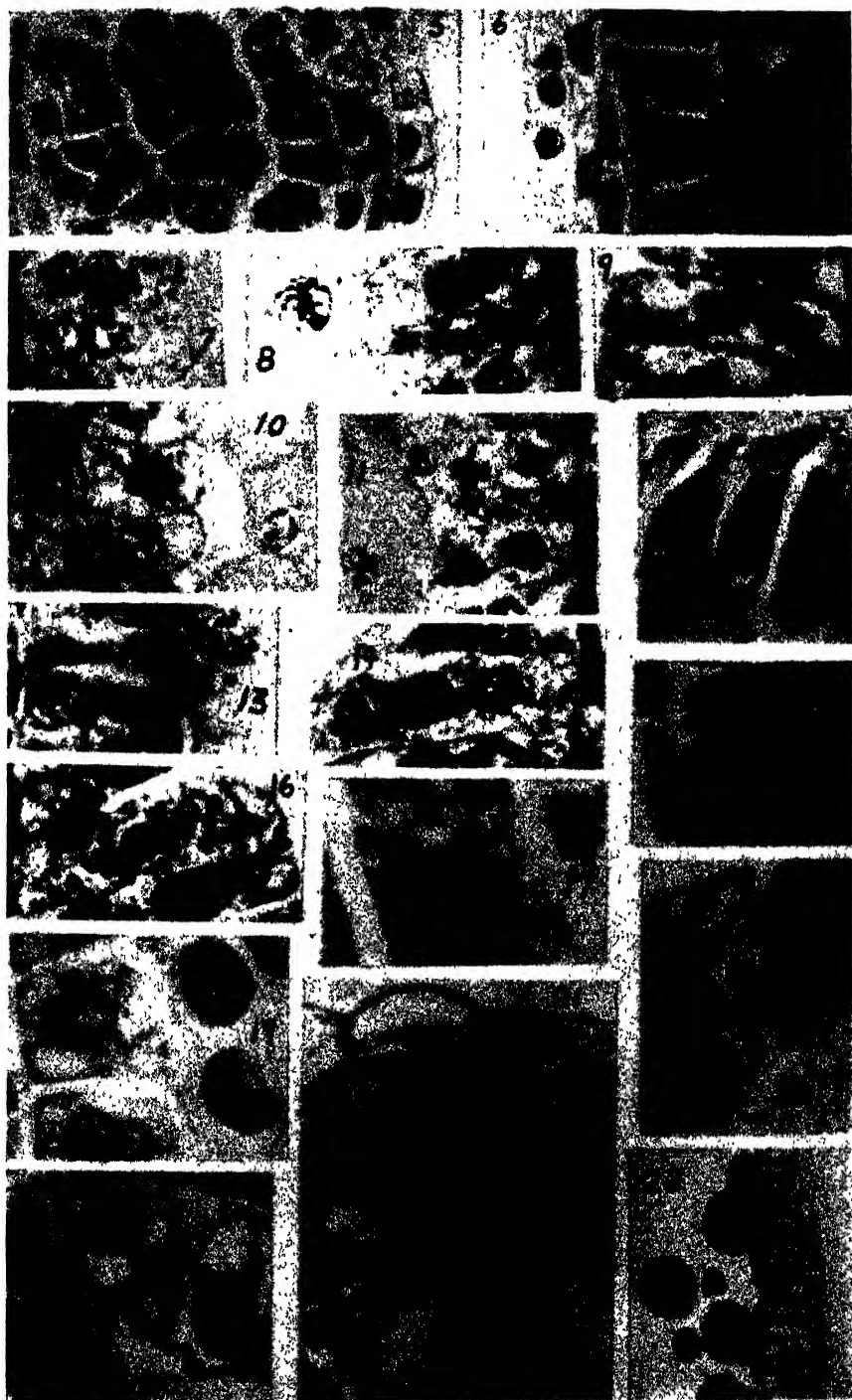
Fig. 11.   Equatorial plates of a second division with the spindles end to end along the longitudinal axis of the cell.   (See text fig. 3.)   This condition results in an anaphase configuration like that shown in figure 8.   × 500.

Fig. 12.   Two tapetal cells, each with one tetraploid and two diploid nuclei resulting from conditions like those shown in figures 8, 9, and 14.   The center cell has four diploid nuclei.   × 500.

Fig. 13.   Tetraploid groups of anaphase chromosomes resulting from the conditions shown in figures 7 and 10.   × 500.

Fig. 14.   Telophases of a second division following an anaphase configuration like that shown in figure 8.   × 750.

Fig. 15.   A tapetal cell with four diploid nuclei following a second division in which two poles of separate spindles did not coincide.   The cell above this has two tetraploid nuclei.   × 500.

Fig. 16.   A third division in a three-nucleate cell.   Only two chromosomes are visible from the nucleus in the left end of the cell.   The center nucleus is in the late prophases and the one at the right is in the equatorial-plate stage.   × 750.

Fig. 17.   A third division of two tetraploid nuclei with the spindles end to end in a diagonal position in the cell.   × 500.

Fig. 18.   A third division of two tetraploid nuclei with the spindles approximately at right angles to each other.   × 500.

Fig. 19.   Two octoploid groups of anaphase chromosomes, following a fusion in the late prophases of two tetraploid nuclei comparable to that of diploid nuclei shown in figure 7.   × 500.

Fig. 20.   Coarsely vacuolate tapetal cells at the time of the homeotypic division in the microspore mother cells.   In the cell at the right two nuclei are pressed together, and these may fuse at a later stage.   Chromatic material is coagulated on the nuclear network.   × 500.

Fig. 21.   Epidermis, endothecium, and tapetum at the time of microspore formation. The nuclei in the tapetal cells are degenerating, and the cytoplasm is densely granular with few vacuoles.   × 500.

Fig. 22.   The breaking loose of tapetal cells from the endothecium at the time of pollen grain formation.   The tapetal cells float out among the pollen grains.   × 85.

THE EFFECT OF SOIL TEMPERATURE ON THE GERMINATION
OF CITRUS SEEDS

A. F. Camp, Harold Mowry, and K. W. Loucks

INTRODUCTION

Aside from a short paper published by Fawcett (1929) on the subject, very few experimental data are available concerning the effect of soil temperature on the germination of citrus seeds. The work reported here has covered a period of several years, from 1925 to 1931. The data on sweet orange, sour orange, rough lemon, and two grapefruit seed experiments were obtained during 1930 and 1931 by the two senior authors; and the remainder of the work on grapefruit was carried on by the junior author incidental to experimental work on citrus canker during the period from 1925 to 1929. A greater number of experiments might result in a more accurate determination of the maximum, minimum, and optimum temperatures; but the greater accuracy probably would not have a greatly increased value from a practical standpoint.

METHODS AND MATERIALS

The work was carried out in the soil tank described by Camp and Walker (1927) and in a smaller unit of similar description in use in the Citrus Canker Research Laboratory (Loucks, 1930). The soil used was sterilized to kill any fungi or other organisms which might cause injury to the seedlings, carefully mixed and potted in 6-inch metal cans (Camp and Walker, 1927), all of the cans being made up to the same weight (soil and cans weighed together). The seeds were planted about 1 inch deep, and the surface of the soil was covered with a 1-inch layer of clean granulated cork ("doll cork"), to check evaporation and to act as an insulator against the loss or absorption of heat. As a general rule the seeds were planted as soon as they were removed from the fruit, since dried seeds germinate more slowly and irregularly than do freshly removed seeds, and if dried too much their viability is destroyed. Irregularity in germination is probably complicated also by the difficulty of getting uniform drying in batches of seed.

The pots were weighed daily and the loss in weight was made up with tap water. Soil temperatures were recorded daily by means of a thermometer thrust into the soil ($1\frac{1}{2}$ to 2 inches). The temperatures shown in the tables are the average temperatures for the duration of the experiment. As has been explained elsewhere, there is considerable variation from the average temperature between day and night as well as from day to day, due to the

variation in the insolation (Camp and Walker, 1927). The temperature of the air also plays a part in this variation, and even with the cork covering over the surface the variation in soil temperature is still considerable. While the temperatures are given to the first decimal place in the tables, a variation of one degree either way from these figures doubtless occurred at times. Such variations can be avoided only by uniform control of both light and heat. The equipment used by the junior author was housed in a laboratory, and the consequent variation in environmental conditions surrounding the soil tank was considerably less than for the experiments carried out in the greenhouse with the larger soil tank. The variation in the temperature ranges in different experiments is due partly to changes in the adjustment of the equipment and partly to variations in weather conditions.

Daily records were made of the first appearance of the seedlings above the soil surface. ━This is not strictly a record of germination, but it was the best that could be obtained under the experimental conditions provided for study under soil conditions. For convenience in this paper this will be termed "emergence" rather than germination. At the conclusion of each experiment the soil in the pots was removed and the remaining seeds were examined. This is important, since some of the experiments could not be prolonged until all of the viable seeds had germinated at the lower temperatures, and it gives an idea as to the number of additional seedlings that might have emerged if a longer period of observation had been allowed. The results of these examinations are reported in the text.

Seeds of the following species and varieties of citrus were studied: sour orange seedling (*Citrus Aurantium* Linn.); sweet or round orange (*Citrus sinensis* Osbeck), variety St. Michael's Blood (exp. 1), variety Valencia (exp. 2), and seedling (exp. 3 and 4); rough lemon seedling (*Citrus Limonia* Osbeck); and grapefruit (*Citrus paradisi* Macf.), seedlings and miscellaneous varieties. The diversity in the sources of grapefruit seeds was due to the fact that fruit was bought on the local market for the citrus canker work and consequently could not be absolutely identified; and in the case of later experiments by the senior authors, no grapefruit seeds were available locally and seeds were obtained from outside sources. These were from "Florida Common" grapefruit, a name generally signifying that the trees present in a planting are budded from good seedling trees or are seedling grapefruit trees.

The data obtained were difficult to present because of the great variation in the time required for emergence at the same temperature in any one experiment, and also because of the great variation between experiments. Various methods of classifying the data based on specified percentages of emergence were tried, but proved to be unsatisfactory. The data as here given cover the average number of days from planting until emergence at each temperature for the seedlings that emerged. This introduces an error at both high and low temperatures, where the percentage of emergence is low

and where more seedlings might have emerged if the period of observation had been prolonged. For convenience in evaluating these errors the percentage of emergence (100 × number of seedlings emerged ÷ number of seeds planted) is recorded for each temperature in each experiment.

EXPERIMENTAL RESULTS

Sweet orange. In table 1 will be found the data for the sweet orange seed experiments. It will be noted that no emergence was obtained in experiment 1 at 16.9° C. and in experiment 2 at 17.6° C. In experiment 4 one seedling emerged, from 40 seeds planted, at 14.7° C., and this seedling did

TABLE I. *The average number of days required for emergence of sweet orange seedlings at various soil temperatures*

Temperature (°C.)	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Av. days	% emergence	Av. days	% emergence	Av. days	% emergence	Av. days	% emergence
14.7							62.0	2.5
16.9	—	None						
17.6			—	None				
18.1					46.2 ± 1.1	63		
20.4	35.0 ± 2.1	10						
20.6					38.9 ± 0.9	73		
21.5			33.0 ± 1.3	25				
23.1	32.5 ± 0.7	27			31.0 ± 0.8	70	25.0 ± 0.6	98
24.2			34.3 ± 0.9	80				
24.5							25.8 ± 0.8	90
25.3					30.7 ± 1.1	68		
25.6	25.7 ± 1.1	37						
26.8			32.7 ± 1.8	30				
27.1							22.0 ± 0.7	93
28.0					26.6 ± 0.7	63		
28.5	25.8 ± 0.9	47						
29.3			30.1 ± 2.0	50				
29.8							22.9 ± 0.8	88
30.4					26.6 ± 1.1	55		
31.4	21.7 ± 1.3	50						
31.7			26.3 ± 1.4	45				
32.4							19.6 ± 0.5	90
33.4					23.6 ± 0.7	78		
34.5	22.3 ± 1.0	67						
34.6			32.6 ± 2.3	25				
35.3							21.5 ± 0.6	78
36.6	—	None						
37.1					27.5 ± 1.4	78		
38.1			30.0	5			--	None
Date planted	3/3/30		4/14/30		1/12/31		3/23/31	
Observation period	40 days		45 days		59 days		66 days	
No. seeds used	30		20		40		40	

not appear until the sixty-second day. Forty-five days after planting, an examination showed that all of the seeds at the above temperatures were dead in experiment 1, and in experiment 2 five were starting to germinate and 15 were dead. In experiment 4, 22 seeds had germinated but failed to

emerge, and 17 were dead. Had the observations extended over a longer period, some of the germinated seeds presumably would have reached the surface, but at temperatures of 15° to 16° C. little or no germination can usually be expected with sweet orange seed. The optimum temperature for emergence seems to lie between 31° and 33° C., but the variations in the results of the different experiments make it impossible to derive an exact figure. In the range of 31° to 33° C., from 19.6 to 26.3 days (averages) were required for emergence. At the higher temperatures it was found in experiment 1 that all of the seeds were dead after 45 days at a temperature of 36.6° C.; in experiment 2 one seed had germinated and 19 were dead after 45 days at a temperature of 38.1° C., and at the same temperature in experiment 4 all of the seeds were dead; while at 37.1° C. in experiment 3, 78 per cent emergence was obtained.

Sour orange. In table 2 will be found the data for three experiments

TABLE 2. *The average number of days required for emergence of sour orange seedlings at various soil temperatures*

Temperature (° C.)	Experiment 1		Experiment 2		Experiment 3	
	Av. days	% emergence	Av. days	% emergence	Av. days	% emergence
14.7					60.3 ± 0.9	43
16.9	—	None				
17.6			40.2 ± 0.5	67		
20.4	31.0 ± 0.9	75				
21.5			30.3 ± 0.6	93		
23.1	25.3 ± 1.2	70			28.1 ± 0.9	80
24.2			26.4 ± 0.9	80		
24.5					25.2 ± 0.9	88
25.6	20.3 ± 1.0	90				
26.8			25.0 ± 0.8	93		
27.1					21.1 ± 0.6	88
28.5	16.2 ± 0.7	80				
29.3			24.8 ± 1.1	87		
29.8					21.1 ± 1.2	70
31.4	16.6 ± 0.7	90				
31.7			21.3 ± 0.9	67		
32.4					18.7 ± 0.5	100
34.5	15.4 ± 0.8	80				
34.6			20.1 ± 1.0	50		
35.3					21.6 ± 1.1	68
36.6	21.6 ± 1.6	45				
38.1			23.8 ± 1.5	27	33.6 ± 1.2	33

	Experiment 1	Experiment 2	Experiment 3
Date planted	3/3/30	4/14/30	3/23/31
Observation period ..	40 days	45 days	66 days
No. seeds planted	20	30	40

with sour orange seed. In experiment 1 no seedlings had emerged in 45 days at a temperature of 16.9°; but when the soil was examined, all of the seeds were found to have germinated. These appeared to be normal and presumably would have emerged had the period of observation been prolonged. In experiment 2, 67 per cent emergence was obtained at 17.6° C.,

and only 5 seeds in 30 were found to be dead. In experiment 3, 43 per cent emergence was obtained at 14.7° C., with an average of 60 days for emergence, while of the remaining seeds, 11 had germinated and 12 were dead. The minimum temperature at which germination and emergence can occur is below 14.7° C., and possibly considerably lower. The optimum soil temperature for germination appears to lie between 32° and 34° C., with an average time for emergence of the seedlings of 15 to 20 days. At the higher temperatures 24 and 34 per cent emergence was obtained at 38.1° C. in experiments 2 and 3, but 18 out of 30 (60 per cent) and 27 out of 40 (67.5 per cent) of the planted seeds, respectively, were found to be dead when the soil was examined.

Rough lemon. In table 3 will be found the data for two experiments

TABLE 3. *The average number of days required for emergence of rough lemon seedlings at various soil temperatures*

Temperature (°C.)	Experiment 1		Experiment 2	
	Av. days	% emergence	Av. days	% emergence
14.8			—	None
17.6	42.7 ± 0.6	15		
21.5	33.8 ± 0.5	80		
21.6			32.5 ± 0.4	60
24.2	25.5 ± 0.8	85		
25.0			27.0 ± 0.4	76
26.8	26.1 ± 1.1	75		
27.3			22.7 ± 0.3	68
29.3	22.1 ± 0.8	85		
30.5			21.0 ± 0.3	76
31.7	26.6 ± 2.1	40		
33.0			19.3 ± 0.02	80
34.6	15.0 ± 0.3	45		
36.0			21.4 ± 0.4	46
38.1	32.8 ± 1.2	40		
38.6			—	None

Date planted 4/14/30		6/5/31
Period of observation .... 45 days		61 days
No. seeds used 20		80

with rough lemon seed. At the lower temperatures 15 per cent emergence was obtained in 45 days (average) at 17.6° C., and of the remaining seeds 11 had germinated and 6 were dead; at 14.8° C. no plants had emerged in 66 days, and 14 seeds were found to have germinated and 18 were dead. The optimum soil temperature probably is close to 34° C. but cannot be accurately determined from the data. At 38.1° C. a 40 per cent emergence was obtained and all of the remaining seeds were dead; and at 38.6° C. all

of the seeds were dead and decayed within three weeks from the date of planting.

Grapefruit. In table 4 will be found the data for two experiments with grapefruit seed. At the lower temperatures a 20 per cent emergence was obtained at 16.9° C., and all of the remaining seeds had germinated and

TABLE 4. *The average number of days required for emergence of grapefruit seedlings at various soil temperatures*

Temperature (° C.)	Experiment 1		Experiment 2	
	Av. days	% emergence	Av. days	% emergence
16.9	37.1 ± 0.6	20		
17.6			34.9 ± 0.6	80
20.4	25.6 ± 0.6	83		
21.5			29.6 ± 0.8	97
23.1	19.6 ± 0.5	97		
24.2			25.0 ± 0.2	80
25.6	15.6 ± 0.8	93		
26.8			21.0 ± 0.7	90
28.5	13.6 ± 0.6	97		
29.3			22.2 ± 1.0	83
31.4	12.6 ± 0.7	87		
31.7			19.4 ± 1.0	80
34.5	13.9 ± 0.4	90		
34.6			23.3 ± 1.2	77
36.6	—	None		
38.1			—	None

Date planted	3/3/30	4/14/30
Period of observation	45 days	45 days
No. seeds used	30	30

were apparently healthy; in the second experiment an 80 per cent emergence was obtained at 17.6° C., with 4 of the remaining seeds germinated and 2 dead. This would indicate a minimum for germination below 16.9° C. The optimum soil temperature would appear to fall between 31° and 33° C., the plants emerging in the shortest time at 31.4° and 31.7° C. in the two experiments, 13 and 19 days from planting, respectively. At the higher temperatures very definite results were obtained: at 36.6° C. in the first experiment all of the seeds germinated, but the seedlings did not emerge; instead they curled about the seed and finally died; at 38.1° C. in experiment 2 none of the seeds germinated. This would indicate a maximum soil temperature for emergence of about 36° C. for the seeds used.

The data obtained on germination of grapefruit seeds in connection with the citrus-canker experiments are shown in table 5. The data from these experiments are difficult to correlate because of the variation in variety of the seeds and the small numbers used in each experiment, usually 10 seeds being planted at a single temperature. Owing to the small numbers, no attempt has been made to give the probable error of the mean. The variation is partly due to the fact that, owing to the small numbers used, an abnormal

TABLE 5. *The average number of days required for emergence of grapefruit seedlings at various soil temperatures (Citrus canker experiments)*

Temperature (°C)	Experiment 1		Experiment 2*		Experiment 3*		Experiment 4		Experiment 5		Experiment 6		Experiment 7		Experiment 8		Experiment 9		Experiment 10		Experiment 11	
	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.	Av. days	% emerg.
13																						
15																						
17													43.2	50	45.0	100						
18			41.5	60	45.4	80	84.0	10			50.6	60										
19							43.4	70					43.2	40	26.4	80			30.0	10		
20			41.1	100	37.7	100	29.7	90			35.0	100					25.0	10			7.6	90
21																						
22			35.5	100					22.2	50												
23	23.8	80											28.6	80	21.7	100						
24					33.1	90	22.0	100											23.2	95		
26			29.0	100	30.0	100			18.7	100	31.6	100	22.4	70			20.0	90			24.4	100
27	19.4	90	22.8	100	22.3	100																
28							15.9	100	14.6	100	24.2	100			17.1	80	16.8	90			19.8	100
29			19.7	10															17.5	95		
30					19.1	100																
31													16.8	80								
32	14.8	50			18.1	90	23.0	60	14.0	100	30.8	100										
33													14.7	100	14.5	100	14.4	100				
34																			18.8	100	16.1	100
36									11.6	100												
38															15.7	90	13.5	100				
39																			23.7	95	34.5	20
44																	—	None	—	None		
Date planted	1/22/25		1/27/25		2/26/25		12/2/25		2/28/26		12/1/26		12/27/26		1/12/27		2/28/27		3/29/27		9/30/27	
No. seeds used	10		10		10		10		10		5		10		10		10		20		10	

* Experiment carried out in large soil tank in greenhouse.

ehavior of only one seed in a lot may upset the figures. An example of this occurs in experiment 4: at 28° C. it was only 9 days from the time the first seedling emerged until the tenth emerged (10 seeds to a lot); while at 24° C. 9 seedlings had emerged within a similar period, but the tenth did not emerge until 15 days later. If only 9 seedlings were to be considered at the latter temperature, the average number of days would be 20, but when the tenth is included it raises the average to 22 days. This accounts in large measure for irregularities within single experiments. In experiment 3 one seedling appeared after 84 days at 13.0° C., and in experiment 7 at the same temperature 50 per cent emergence (5 seeds out of 10) was obtained, with an average of 43.2 days. In experiment 8, 100 per cent emergence was obtained at 15° C., with an average of 45 days required. The data with regard to the optimum are so irregular as to make it almost impossible to draw valid conclusions. By grouping the material, it would appear that the optimum soil temperature would fall between 32° and 34° C., which agrees very well with the other experiments reported here. The data with regard to the maximum temperature are considerably at variance with those obtained in the other experiments reported. In experiment 10 a 95 per cent emergence was obtained at 38° C., and in experiment 11 a 20 per cent emergence at 39° C. Seed failed to germinate at 44° C. in experiment 10. Whether the difference in maximum obtained in these experiments as compared with the two experiments reported above was due to the seeds used or to operating conditions could not be determined. It is possible that the difference may have been due in part to the fact that the soil tank was in the laboratory rather than in the greenhouse, thus giving rise to fewer and smaller temperature fluctuations in the soil.

DISCUSSION

It is difficult to analyze the foregoing data in any exact way, though the general relationships seem clear. It would appear as though grapefruit and sour orange seeds would germinate at slightly lower soil temperatures than those of sweet orange and rough lemon. This relationship is made somewhat clearer if the time required for emergence at 20° C. is noted. In general it might be pointed out that below 20° C. the time required for emergence of seedlings is very long as compared with higher temperatures, and the planting of citrus seeds in soils where the temperature is below 20° C. is of doubtful value.

To determine the optima from the data with a high degree of accuracy is impossible. The figures given in the report of the experimental work are approximations derived from careful analyses of the data. These estimates would indicate that sour orange and rough lemon seeds have slightly higher optima than do the seeds of grapefruit and sweet orange, though an increased number of experiments might show these relationships to be only approximately correct.

It seems clear that the maximum temperature lies below 40°. C., thoug, very near to it. This would agree with Fawcett (1929), who reported no growth at 104° F. (40° C.). There is some difference between the two sets of grapefruit experiments, the work on citrus canker indicating a somewhat higher maximum temperature than do the other experiments, though this difference is not great. It is apparent that above 35° C. the rate of growth of the seedlings and the germination of the seeds are retarded.

The trends in the curves between the minimal temperatures and the optimal ranges are found to agree well with the Van't Hoff-Arrhenius rule, and this is to be expected. No data are reported on the growth of these seedlings, though some were taken. As was the case with cotton (Camp and Walker, 1927), it was found that other factors than the soil temperatures were active as limiting factors after the plants were up (Blackman, 1905). To what extent the optimum for germination may differ from the optimum soil temperature for growth remains to be determined, but the indications are that there is little difference between the optima for these processes. After the plant has emerged from the soil, many factors affect it, so that it is difficult to differentiate between soil and air effects. In Fawcett's (1929) experiments there is an indication that after aërial growth has started a lower optimum is indicated. This same relationship was indicated in these experiments, the best seedlings being found at temperatures of 28° to 30° C. Considering the work of Balls (1919), it is not surprising that such an apparent reduction in optima should be obtained. The explanation for this might be that with increased time the toxic products accumulate at the temperatures from 30° to 35° C., and a slowing down in growth at these temperatures is apparent, though this effect is not noted in the germination.

SUMMARY

Experiments carried out under controlled conditions to determine the effect of soil temperature on the germination of citrus seed are reported. These experiments covered four citrus species, as follows: grapefruit (*C. paradisi* Macf.); sweet orange (*C. sinensis* Osb.); rough lemon (*C. Limonia* Osb.); and sour orange (*C. Aurantium* Linn.).

Considerable variation in the time required for germination was found to occur within any single lot of seeds held at the same soil temperature and among different batches of seeds planted at different times. Because of such variations, which presumably are normal, it was not possible to define with absolute accuracy the minimum, maximum, and optimum soil temperatures for germination. It was found that minimum temperatures for all species were probably below 15° C., the maxima a little below 40° C., and the optima between 31° and 35° C. Grapefruit and sweet orange appear to have slightly lower optima than sour orange and rough lemon, though this relation has not been absolutely established.

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NUCLEAR DIVISIONS IN THE TAPETAL CELLS OF CERTAIN ANGIOSPERMS

D. C. COOPER [1]

Conflicting statements are to be found in the literature concerning the nature of the nuclear divisions in the tapetal cells of angiosperm pollen sacs. A number of investigators have made casual observations concerning these divisions in the plant with which they were working; and in a few instances, notably by Tischler (1905), Bonnet (1912), and Mascré and Thomas (1930), detailed studies of the divisions of the tapetal nuclei have been made.

The earlier workers reported fragmentation, or amitosis, as the typical method of nuclear division in the cells of the tapetum. Strasburger (1882), reëxamining his preparations, found mitotic figures in the tapetal cells and concluded that the appearances formerly described by him as amitosis result from the fact that two daughter nuclei remain attached and finally come to lie in close contact. Tischler (1905) in a study of three species of *Ribes* reported that the first division of the tapetal nucleus is by mitosis and that any further division is by constriction or fragmentation. Bonnet (1912), after critically examining the tapetal cells of ten species of angiosperms, concluded that amitosis does not occur in the nuclei of the tapetal cells and that figures suggesting amitosis are due to mitotic irregularities and nuclear fusions. In one of the plants studied by Bonnet, *Datura Stramonium*, O'Neal (1920) reported that the nuclei of the tapetum divide by fragmentation. Mascré and Thomas (1930) agree with Bonnet concerning the absence of amitosis, but find that the tapetal cells may again become uninucleate through nuclear fusions.

The presence of typical mitoses in the tapetal cells of *Buginvillaea glabra* (Cooper, 1931) suggested an examination of other angiosperms with the idea of determining, if possible, the exact nature of these nuclear divisions. Material of 7 monocotyledons and 36 dicotyledons, representing in all 24 families, was collected and examined. With the exceptions of the preparations of *Pereskia aculeata*, which were furnished by Dr. R. I. Evans, and those of *Lilium canadense, Ranunculus fascicularis, Menispermum canadense*, and *Gossypium barbadense*, which were loaned by Dr. E. L. Fisk, the material for this study was collected in the botanical greenhouses or about the campus of the University of Wisconsin. Flemming's medium solution was used as a

fixative, the material was embedded in paraffin, and microscopic preparations were made in the usual manner.

OBSERVATIONS AND DISCUSSION

With reference to nuclear phenomena, the tapetal cells of the species examined fall in three roughly separable groups: (1) those in which the nucleus rarely, if ever, divides and the cell remains uninucleate; (2) those in which the nucleus divides mitotically once at about the time when the neighboring pollen mother cells are in synizesis and the tapetal cell remains binucleate thereafter; and (3) those in which the nucleus first divides as in the second group, after which time further mitoses may occur resulting in plurinucleate cells, or through incomplete divisions the cells may remain uninucleate or binucleate. Considerable overlapping, however, is found between these groups.

The cells in the tapetum of four species of *Medicago*—namely, *M. sativa, M. platycarpa, M. glutinosa,* and *M. ruthenica*—and in two species of *Melilotus—M. alba* and the variety, Redfield yellow—remain constantly uninucleate, and no division figures were observed in them at any stage. Reeves (1930) noted that the cells of the tapetum of alfalfa remain uninucleate, and Maheshwari (1931) found a similar condition in *Albizzia lubbck,* a member of the Mimosaceae.

No division figures were found and the uninucleate condition was constant in the tapetum of *Delphinium Ajacis.* In contrast, nuclear divisions were abundant and the binucleate condition was found characteristic of the later stages in *Ranunculus fascicularis* (fig. 23, pl. 16), another member of the same family. Coulter (1898) reported that the cells of the tapetum of *Hepatica acutiloba* contain from two to thirteen nuclei.

The uninucleate condition is almost constant in *Phlox paniculata,* but occasionally binucleate cells are to be found. Mitotic figures were observed in a few cells at about the time the pollen mother cell nuclei were in diakinesis or in the heterotypic metaphases, but no divisions were to be seen at earlier stages in the tapetal cells.

In all other species examined, the first division of the tapetal nucleus takes place at about the time the pollen mother cells are passing into or through the stages of synizesis. In many species (table 1) there is a condition similar to

TABLE I. *List of species studied in which the division of the tapetal nucleus takes place at about the time of synizesis in the pollen mother cells, and the tapetal cells remain binucleate thereafter.*

Sansevieria zeylanica (fig. 15, pl. 16)
Ulmus americana (fig. 18, pl. 16)
Pellionia Daveauana (fig. 19, pl. 16)
Oxybaphus nyctagineus (fig. 21, pl. 16)
Buginvillaea spectabilis (fig. 22, pl. 16)
Ranunculus fascicularis (fig. 23, pl. 16)
Menispermum canadense (fig. 24, pl. 16)
Capsella Bursa-pastoris (fig. 30, pl. 17)
Euphorbia splendens (fig. 33, pl. 17)
Althaea rosea (fig. 34, pl. 17)
Gossypium barbadense (fig. 35, pl. 17)

Shepherdia canadensis (fig. 37, pl. 17)
Primula sinensis (fig. 38, pl. 17)
Coleus Blumei (fig. 39, pl. 17)
Nepeta hederacea (fig. 40, pl. 17)
Lycopersicon esculentum (fig. 42, pl. 17)
Petunia hybrida (fig. 43, pl. 17)
Digitalis purpurea (fig. 45, pl. 17)
Campsis radicans (fig. 46, pl. 17)
Plantago major (fig. 47, pl. 17)
Lonicera tartarica (fig. 48, pl. 17)

that already reported for *Buginvillaea glabra* (Cooper, 1931) in which the tapetal cells remain binucleate after this one division. The nuclei may come to lie closely appressed to each other so as to give the illusion of a stage in amitosis, or their adjacent surfaces may become so flattened that there is the appearance of a single nucleus. It is possible that these nuclei actually fuse at later stages, as has been reported by Mascré and Thomas (1930). No cases, however, were observed in which the majority of the tapetal cells were uninucleate in the later stages, excepting in those species in which the nuclei divide rarely or not at all.

Certain chromosomes may fail to disjoin in the anaphases of the first or succeeding mitoses, and the two united chromosome groups then form a dumbbell-shaped, tetraploid nucleus whose appearance suggests a stage in direct division. Such non-disjunction and resulting united nuclei were occasionally observed in *Lilium canadense* (fig. 11–13, pl. 16), *L. Henryii, Hosta caerulea, Podophyllum peltatum* (fig. 25, 26, pl. 16; fig. 27–29, pl. 17), *Nepeta hederacea* (fig. 40, pl. 17), and *Plantago major* (fig. 47, pl. 17). In the two latter species, however, this condition is very rare, and the mature tapetal cells are usually binucleate. Polar views of later divisions of such united nuclei show an increased number of chromosomes. Twenty-four chromosomes (tetraploid) could be counted in a number of polar views of anaphase figures in the tapetal cells of *Podophyllum peltatum*, and in one figure more than 40 chromosomes (octoploid?) were present. Winkler (1906) noted that polyploid nuclei are present in consequence of nuclear fusions in the tapetal cells of *Wikstroemia indica*, and Smith (1933) has found tetraploid and octoploid nuclei in *Galtonia candicans*. Tahara (1905) reported " syndiploid " nuclei in the tapetal cells of *Morus*, and Bonnet (1912) found fusion nuclei of high valence in the plants with which he worked.

The tapetal nuclei of many species may divide further, the cells thus becoming plurinucleate (table 2). The second division usually occurs at about the time that the nuclei of the pollen mother cells are at stages ranging from early spireme to diakinesis and in rare instances as late as the heterotypic anaphases. Four nuclei are occasionally to be found in a tapetal cell in *Zea mays, Rhoeo discolor, Lilium canadense, L. Henryii, Yucca filamentosa, Polygonum persicaria,* and *Pyrus americana.* In some plants a four-nucleate condition is typical of the mature tapetal cell, as in *Lactuca scariola* (Gates and Rees, 1921). Further divisions occur in the tapetum of *Taraxacum officinalis,* and the mature cells have either 8 or 16 nuclei.

Three as well as four nuclei were occasionally observed in tapetal cells of *Rhoeo discolor, Lilium canadense, L. Henryii, Yucca filamentosa,* and *Lycium halimafolium.* In such instances the central nucleus is usually considerably the largest of the three. The production of these three nuclei may be the result of an orientation of the two spindles of the second division in such a way that two of the poles are closely adjacent, as is shown in figure 17 of plate 16 for *Yucca filamentosa.* The chromosomes at the adjacent poles then

TABLE 2. *Species studied in which the early division of the tapetal nucleus is followed by further divisions or irregularities*

Species	Synizesis of pollen mother cells	Contemporaneous phenomena in tapetal cells	
		Open spireme to diakinesis of pollen mother cells	Heterotypic division of pollen mother cells to microspores
Zea Mays (Fig. 1–8, pl. 16)	First nuclear division	Few second divisions	Usually binucleate, rarely tetranucleate
Rhoeo discolor (Fig. 9, pl. 16)	First nuclear division	Few second divisions	Mostly binucleate; some cells with three or four nuclei
Hosta caerulea (Fig. 10, pl. 16)	First nuclear division, often incomplete	Later divisions, often incomplete	Mostly binucleate; cells with one incompletely divided nucleus as well as 3- and 4-nucleate cells are present
Lilium canadense (Fig. 11–13, pl. 16)	First nuclear division, often incomplete	Some second divisions	Mostly binucleate; cells with one incompletely divided nucleus as well as 3- and 4-nucleate cells are present
Lilium Henryii (Fig. 14, pl. 16)	First nuclear division	Some second divisions	Binucleate, rarely tri- or tetranucleate
Yucca filamentosa (Fig. 16, 17, pl. 16)	First nuclear division	Some second divisions	Binucleate, rarely tri- or tetranucleate
Polygonum persicaria (Fig. 20)	First nuclear division	Few second divisions	Binucleate, rarely tetranucleate
Podophyllum peltatum (Fig. 25, 26, pl. 16; fig. 27, 29, pl. 17)	First nuclear division, often incomplete	A few second divisions giving polyploid nuclei	Uninucleate and binucleate
Pyrus americana (Fig. 31, 32, pl. 17)	First nuclear division	Occasional second divisions	Binucleate, rarely tetranucleate
Pereskia aculeata (Fig. 36, pl. 17)	First nuclear division	Second divisions	Usually binucleate. Cells often contain 3 or 4 nuclei
Lycium halimifolium (Fig. 36, pl. 17)	First nuclear division	Second divisions	Usually binucleate; rarely cells contain 3 or 4 nuclei
Antirrhinum majus (Fig. 44, pl. 17)	First nuclear division	Few second divisions	Mostly binucleate; rarely tetranucleate
Lactuca scariola (Fig. 49, pl. 17)	First nuclear division	Second divisions	Usually tetranucleate
Taraxacum officinalis	First nuclear division	Later divisions	Cells contain 8 or 16 nuclei

unite into a common tetraploid nucleus, and the chromosomes at the two opposite poles form diploid nuclei. An analogous condition was found by Smith (1933) in *Galtonia candicans*. The three-nucleate condition may also possibly result from division of only one of the two nuclei formed by the first division, but such an occurrence was not observed. If this does happen, however, all three nuclei would be diploid and approximately similar in size.

SUMMARY

1. Nuclear division in the tapetal cells of the 35 species of angiosperms examined is mitotic.

2. The 43 species studies can be grouped in three classes according to the behavior of the tapetal nuclei: (*a*) the nucleus does not divide, and the mature tapetal cell is uninucleate; (*b*) the nucleus divides once, and the mature cell is binucleate; (*c*) the nucleus divides more than once, and depending upon the incompleteness or completeness of the divisions, the mature cell may be uninucleate, binucleate, or plurinucleate.

3. Amitotis-like figures observed in the mature tapetal cells of some plants were found to result from incomplete mitoses.

4. A multinucleate condition was found to be characteristic of the mature tapetum of *Lactuca scariola* and *Taraxacum officinalis*.

5. The presence of tetraploid and octoploid nuclei was occasionally noted.

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EXPLANATION OF PLATES

All drawings were made with an Abbé camera lucida at table level. Spencer compensating oculars and a Leitz oil immersion objective N. A. 1.32 were used. The magnification is given for each figure. All drawings are of the first division of the nucleus of a tapetal cell unless otherwise noted.

PLATE 16

Fig. 1–7. *Zea Mays*. Stages in the division of the tapetal nucleus. × 1450.

Fig. 8. *Z. Mays*. Equatorial plate stage, second division. × 1450.

Fig. 9. *Rhoeo discolor*. Late anaphase. × 775.

Fig. 10. *Hosta caerulea*. Late anaphase. × 775.

Fig. 11. *Lilium canadense*. Anaphase. × 400.

Fig. 12. *L. canadense*. Telophase showing the daughter nuclei connected by non-disjoined chromosomes. × 775.

Fig. 13. *L. canadense*. Resting stage showing dumbbell-shaped nucleus resulting from non-separation of chromosomes. × 775.

Fig. 14. *L. Henryii*. Anaphase. × 775.

Fig. 15. *Sansevieria zeylanica*. Late telophase. × 1450.

Fig. 16. *Yucca filamentosa*. Anaphase. × 775.

Fig. 17. *Y. filamentosa*. Telophase of second division. × 775.

Fig. 18. *Ulmus americana*. Late metaphase. × 1450.

Fig. 19. *Pellionia Daveauana*. Metaphase. × 1450.

Fig. 20. *Polygonum persicaria*. Metaphase. × 1450.

Fig. 21. *Oxybaphus nyctagineus*. Metaphase. × 1450.

Fig. 22. *Buginvillaea spectabilis*. Metaphase. × 1450.

Fig. 23. *Ranunculus fascicularis*. Late anaphase. × 1450.

Fig. 24. *Menispermum canadense*. Metaphase. × 1450.

Fig. 25. *Podophyllum peltatum*. Early anaphase. × 775.

Fig. 26. *P. peltatum*. Late anaphase showing non-separation of one pair of chromosomes. × 1450.

PLATE 17

Fig. 27, 28. *Podophyllum peltatum*. Late telophase; chromosomes extending between daughter nuclei. × 1450.

Fig. 29. *P. peltatum*. Amitotis-like figure consequent upon incomplete mitosis. × 1450.

Fig. 30. *Capsella Bursa-pastoris*. Metaphase. × 1450.

Fig. 31. *Pyrus americana*. Metaphase. × 1450.

Fig. 32. *P. americana*. Metaphase of second division. × 1450.

Fig. 33. *Euphorbia splendens*. Telophase. × 1450.

Fig. 34. *Althaea rosea*. Telophase. × 1450.

Fig. 35. *Gossypium barbadense*. Telophase. × 1450.

Fig. 36. *Pereskia aculeata*. Telophase. × 1450.

Fig. 37. *Shepherdia canadensis*. Metaphase. × 1450.

Fig. 38. *Primula sinensis*. Early telophase. × 1450.

Fig. 39. *Coleus Blumei*. Metaphase. × 1450.

Fig. 40. *Nepeta hederacea.* Telophase showing connecting strand between daughter nuclei. × 1450.

Fig. 41. *Lycium halimifolium.* Metaphase. × 1450.

Fig. 42. *Lycopersicon esculentum.* Metaphase. × 1450.

Fig. 43. *Petunia hybrida.* Telophase. × 1450.

Fig. 44. *Antirrhinum majus.* Metaphase. × 1450.

Fig. 45. *Digitalis purpurea.* Metaphase. × 1450.

Fig. 46. *Campsis radicans.* Telophase. × 1450.

Fig. 47. *Plantago major.* Telophase showing connection between daughter nuclei. × 1450.

Fig. 48. *Lonicera tartarica.* Metaphase. × 775.

Fig. 49. *Lactuca scariola.* Metaphase of second division. × 1450.

27　　28　　29　　30　　31　　32　　33　　34　　35　　36　　37　　38　　39　　40　　41　　42　　43　　44　　45　　46　　47　　48　　49
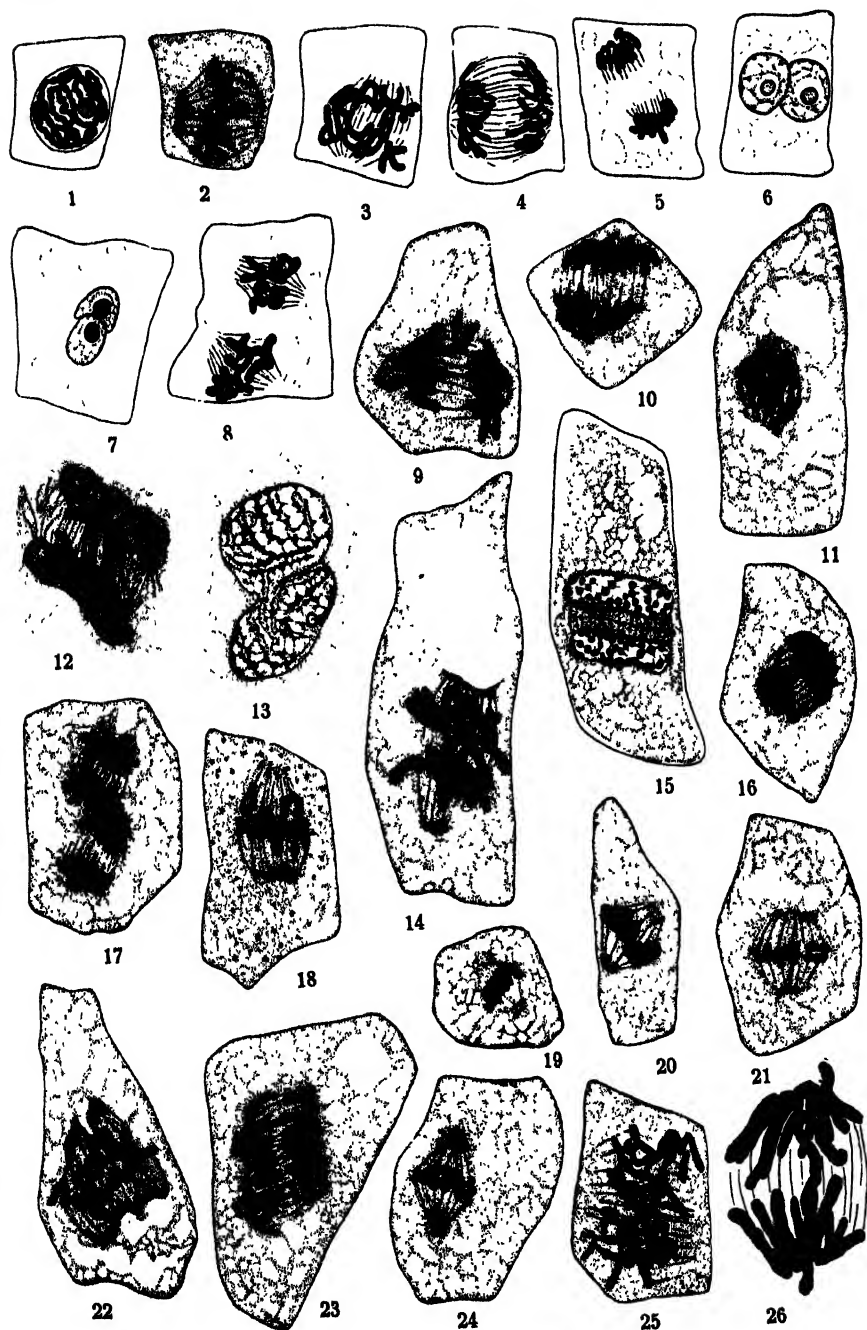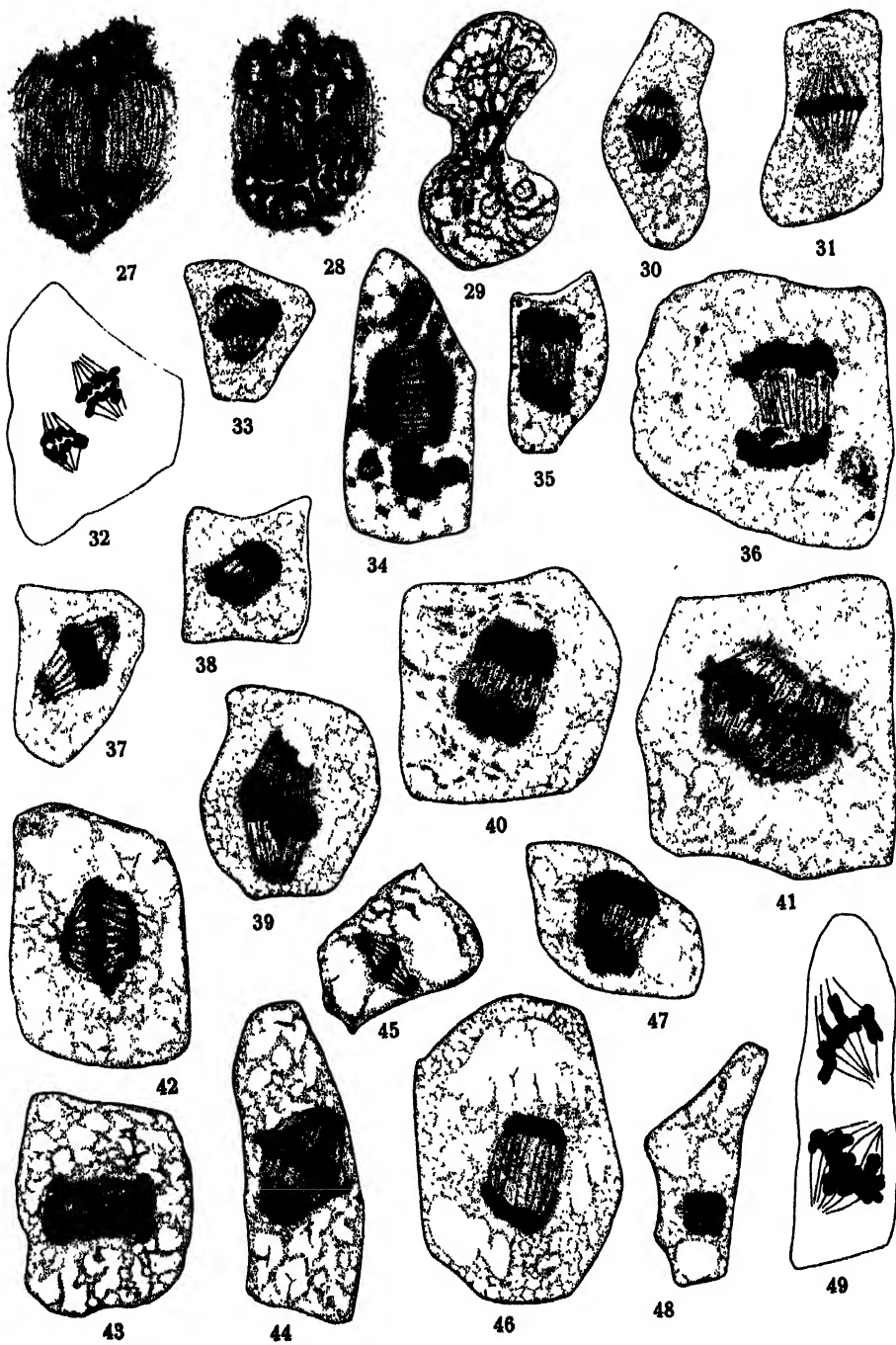
COOPER: TAPETAL NUCLEI

ORGANIZATION AND DEVELOPMENT OF FOLIAR ORGANS IN *PAEONIA OFFICINALIS*[1]

ADRIANCE S. FOSTER AND FRED A. BARKLEY

INTRODUCTION

A fundamental characteristic of shoot development in the higher plants is the repetitive formation of foliar primordia by the growing point. As is well known, the foliar initials, according to their relative positions, experience specific differentiation, a process often leading to a distinct seriation of adult foliar organs differing in form, organization, and function. Although many of the general aspects of this " open type of embryology " are well known (cf. Foster, 1928), the more difficult question of the underlying causes of foliar differentiation in plants remains unanswered (cf. Schüepp, 1929, p. 800). It seems obvious that any sound interpretation of this problem must inevitably rest upon extensive researches of a comparative as well as a developmental-experimental character.

Previous studies of the senior author (Foster, 1929, 1931b, 1932a, 1932b) on *Aesculus Hippocastanum* L. and *Carya Buckleyi* var. *arkansana* Sarg., those of Schüepp (1929) on *Acer pseudoplatanus* L., and Diels' (1930) suggestive experiments on *Asarum europaeum* L. have dealt entirely with the problem of cataphyll and foliage-leaf differentiation. The relation of these investigations to the problem of organ determination has already been critically examined (Foster, 1931a, p. 246–248; 1932a, p. 92–93) and requires no discussion here. During the past two years a joint investigation has been made by the writers of the organization and development of cataphylls and foliage leaves in *Paeonia officinalis* L. This plant was selected for study because, in common with many herbaceous perennials, the annual shoots exhibit a marked seriation of different leaf types. In particular, the cataphylls and foliage leaves are strikingly divergent at maturity and are thus well suited for ontogenetic and experimental study.

The data and conclusions presented in this paper have resulted from joint research by both writers. The illustrations were exclusively prepared by the junior author.

MATERIALS AND METHODS

The plants used for this investigation were largely of the horticultural variety Zoe Callot and were obtained from the Noble Nursery, Noble, Okla-

37

homa.   A preliminary study of bud material at various dates indicated the desirability of frequent collections.   Material was therefore obtained the first and fifteenth of each month from July to November, 1931.   Subsequent collections were made December 1, 1931, March 15, March 20, March 24, March 28, and April 3, 1932.   Ontogenetic and morphological studies were made on the largest buds on each tiller.   Because of varying field conditions and the numerous plants used, it was very difficult to secure complete developmental stages of the various foliar organs.

All of the material was preserved in 5 per cent formalin solution, which toughens the tissues sufficiently for purposes of dissection.   For an accurate study of venation the cataphylls were removed from the formalin solution, washed in water, and placed in a concentrated solution (5 to 1) of chloral hydrate.   The ventral epidermis was then carefully removed, and the cataphylls were placed between slides and flooded with chloral-hydrate solution for examination.   In the ontogenetic studies the buds were prepared for observation and dissection according to a method previously published (Foster, 1932b, p. 718–719).

All detailed observations were made with a Spencer dissecting microscope at magnifications ranging from 39.1 to 115.6 diameters.   The drawings of primordia and vein systems were made with the aid of a camera lucida.

OBSERVATIONS

Origin and organization of the shoot system

Paeonia has a sympodial type of shoot system.   Tillers developing from subterranean buds on the bases of the last year's, or previous years', shoots form the annual aerial portion of the plant (fig. 1–4, pl. 18).

Usually there are one or two expanding buds on the bases of each of the past year's vegetative shoots (fig. 1, 4, pl. 18).   However, below these buds there are often two to five smaller buds that remain dormant for a number of years, so that on " clumps " of *Paeonia* obtained during the winter one may find large buds, apparently arising adventitiously, which are in reality the lower dormant buds on basal portions of the annual shoots of former years that simulate roots in appearance because of their position, formation of adventitious roots, and secondary growth (fig. 1, pl. 18).

Usually one bud on the strongest shoots of the previous year is obviously better developed than the others on the shoot (table 1) and is destined to produce the most vigorous vegetative shoot and also the flower.   Rarely do more than three or four buds show indications of becoming blossoming shoots.   On these stronger shoots appear about twenty-one cataphylls, fifteen foliage leaves, and the blossom.   Other buds that are markedly small when compared to the buds just mentioned produce weak shoots with as few as nine cataphylls, six foliage leaves, and an abortive blossom.   Such small shoots rarely blossom.   The conditions just described represent extreme variation in shoot vigor and composition, between which many intermediate types have been ob-

TABLE I. *Dimensions of the series of buds on the axes of annual shoots collected June 18, 1931. The bud on each axis that seems "destined" to produce the following year's tiller is italicized. The upper figure for each bud is the length of the bud, while the lower is the width. The measurements are given in millimeters.*

Subtending cataphyll	Shoot 1	Shoot 2	Shoot 3	Shoot 4	Shoot 5	Shoot 6	Shoot 7
12		Decayed					
11	Decayed	Decayed	Decayed				
10	Decayed	Decayed	Decayed				Decayed
9	Decayed	Decayed	Decayed	Decayed	Decayed	Decayed	Decayed
8	3.00 1.75	Decayed	Decayed	Decayed	Decayed	Decayed	Decayed
7	2.20 1.90	3.00 2.90	Decayed	Decayed	Decayed	Decayed	5.00 2.10
6	*2.80 2.90*	2.30 3.20	*1.80 1.50*	3.80 1.90	Decayed	2.80 1.90	*4.20 4.50*
5	2.75 2.20	2.50 2.60	1.00 .90	*4.25 5.10*	*4.00 3.80*	2.20 1.90	2.50 3.00
4	1.60 1.80	1.80 2.00	1.10 .80	1.70 2.10	2.00 1.80	.90 1.00	1.00 1.30
3	2.00 2.00	1.30 1.50	.80 .80	1.20 1.60	1.80 2.20	1.20 1.20	2.80 2.50
Prophyll	1.10 1.20	.80 .80	.70 .60	1.10 1.50	1.30 1.70	.90 1.10	.75 1.50
Prophyll	1.10 1.10	.50 .40	.20 .20	.80 1.00	.50 .70	.80 .60	.40 .50

served. Typical variations in the foliar organization of the shoot are summarized in table 2.

Buds are usually found developed or developing in the axils of all the cataphylls of the annual shoot (fig. 4, 7, 8, pl. 18). The number of cataphylls of these buds vary, the number decreasing progressively up the stem, until the last two are usually scaleless (fig. 7, 8, pl. 18; table 3). The bud of the last cataphyll is usually less developed than the buds of those preceding. On

TABLE 2. *The vegetative foliar organs of ten expanding buds of Paeonia officinalis L.*

Shoot	Cataphylls	Transitional forms	Foliage leaves	Total
1	8		7	15
2	8		7	15
3	8	1	7	16
4	10		9	19
5	11		10	21
6	14		9	23
7	14		12	26
8	15		13	28
9	17		12	29
10	18		14	32
Average	12.3	0.1	10	22.4

TABLE 3. *The foliar organs found in buds in the axils of the cataphylls on expanding buds. Each vertical line represents the series of buds found on one axis. The first number given indicates the number of cataphylls, while the second refers to the number of foliage leaf primordia. (The lowest foliage leaf of shoot six had a growing point with one foliar primordium differentiated.) In each shoot the last cataphyll's bud was smaller than the last few preceding buds.*

Shoot I	Shoot II	Shoot III	Shoot IV	Shoot V	Shoot VI	Shoot VII	Shoot VIII
			1–0		g p	2–0	g p
			3–0		g p	3–0	g p
			4–0		3–0	2–0	2–0
		g p	6–0		3–0	g p	2–0
2–0	3–0	g p	6–0	4–0	4–0	4–0	3–0
4–2	4–0	g p	2–4	5–0	4–2	5–0	4–0
1–7	1–3	4–0	1–5	6–0	2–3	4–2	4–0
0–5*	0–3*	4–0	0–5	3–2	2–4	4–3	4–3
0–3*	0–2*	1–3*	0–4*	3–3	2–5	6–1	2–3
	0–2*	0–3*	0–3*	4–4	2–3	6–2	2–4
		0–3*	0–2*	2–4	2–3	6–2	2–4
		0–3*	0–2*	2–4	2–3	3–6	2–5
		0–3*	0–2*	2–6*	2–3	2–4*	2–5
		0–2*	0–2*	1–7*	1–4*	2–4*	2–5
				0–5*	1–3*	1–5*	2–4*
				0–2*	0–3*	0–4*	2–4*
				0–2*	0–3*	0–4*	2–4*
				g p	0–3*	0–2*	0–4*
							0–3*
							0–3*
							g p

* Indicates that a flower bud has been formed.

shoots planted so that one bud was above ground, two upper cataphyllary buds produced small lateral branches (fig. 3, pl. 18). Table 1 shows the dimension of the buds still persistent on the shoot in early summer.

In most cases observed the last cataphyll is followed directly by a typical foliage leaf. In certain cases, however, notably where shoots have arisen under abnormal conditions (e.g., where shoots have developed from buds unusually near the surface, or exposed above the ground), the last bud scale is followed by a foliar organ intermediate in character between typical cataphyll and typical foliage leaf (fig. 2–18).

There are between six and fifteen foliage leaves on normal shoots (table 2), the lowest of which is the most highly developed (fig. 4–9, pl. 20). In contrast to the well-organized buds found in the axils of the cataphylls, the foliage leaves merely possess small, raised patches of meristematic tissue which, at least in the variety under consideration, rarely produce foliar organs.

Organization of the axillary bud in winter

In common with many perennials there is a telescoping of bud within bud in *Paeonia*. In June a new generation of buds appears in the axils of the cataphylls of a generation of buds a year older (fig. 9–12, pl. 18). This older

generation of buds in turn was produced in the axils of the cataphylls of the present tiller. In order to understand this complex telescoped foliar cycle of *Paeonia,* it will first be necessary to examine the structure of a winter bud.

In the winter bud of *Paeonia* there are found from nine to twenty-one cataphylls, six to fifteen foliage leaves, and the primordia of the floral organs. Thus, as in the case of many woody plants, all of the foliar organs destined to expand during the spring are recognizable in the resting bud (cf. Moore, 1909; Foster, 1929, 1931b).

The two prophylls and the four succeeding cataphylls are more or less decussately arranged, while the upper scales, like the foliage leaves, are spirally placed with a 2/5 divergence (fig. 1). The cataphylls are imbricate in

Fig. 1. Transverse section of a first generation bud in June, showing the decussate arrangement of the prophylls and the four succeeding cataphylls. The spiral phyllotaxy is initiated by the seventh scale. × 25.

aestivation, and as a result of their continued growth during the winter, form a protected cavity within which is found the loosely arranged cluster of foliage leaves (fig. 7, pl. 18). In some buds the cataphylls are crowded together without distinct internodes, while in other buds the upper scales, at least, are separated by internodal development. Internodal elongation is often evident between the individual foliage leaves of the winter bud and between the last cataphyll and the first foliage leaf (fig. 7, 8, pl. 18).

There is a progressive increase in the size of the cataphylls of the bud (fig. 1–18, pl. 19). The first five or six scales mature early, and when examined during the winter are in various stages of decay. The upper cataphylls increase in length slowly during the winter and vary in color from a waxy white in the unexposed parts to a deep rose in the aerial portions, which may become raised above the ground during bud expansion.

The foliage leaves of the winter bud tend to be valvate in aestivation, although the leaf bases may imbricate to some extent (fig. 8, pl. 18). The vernation of the leaflets of the foliage leaf varies from plane through involute to a condition approaching conduplicate.

In late March and early April (in the vicinity of Norman, Oklahoma) the bud expands into the annual shoot which, if fertile, blooms during May.

The bud cycle in peony

The annual shoot is formed from a bud which normally requires approximately twenty months for its complete development. This relatively long developmental period of the bud in peony occurs as a result of the telescoping of the new generation of buds within the buds formed the previous year. For purposes of convenience in discussion the winter buds destined to expand the following spring are designated as first generation buds. Some of the buds found in the axils of the scales of these winter or first generation buds are destined to expand twenty months later; these buds are designated as second generation buds.

The second generation buds appear externally in June when foliar differentiation begins by the formation of a prophyll (fig. 9–12, pl. 18). Usually these buds begin to appear in the axils of the fourth to eighth cataphylls of the first generation buds. Buds are rarely found in the axils of the prophylls, and often only patches of meristem are found in the axils of the lower cataphylls.

Production of foliar organs continues in the second generation buds during the summer and autumn. By winter fully formed second generation buds with floral organs present are found in the axils of the upper cataphylls of the first generation bud (fig. 7, 8, pl. 18; fig. 18, pl. 21). These either expand the following spring with the first generation bud (fig. 3, pl. 18), or more often abort. The uppermost of the second generation buds is usually much smaller and less developed than the immediately lower buds.

There is a progressive increase in the number of foliar organs in the second generation buds subtended by the middle series of cataphylls of the first generation buds which involves a progressive decrease in the number of cataphylls from the lower buds to the upper buds, from all cataphylls in the lower, to no cataphylls in the upper; and a progressive decrease in the number of foliage leaves from two to four in the uppermost, to none in the lower (at the end of the first season's growth) (table 3). The second generation buds in which foliage leaves are found, and which do not expand with the first generation buds, abort after the expansion of the first generation buds.

Usually one or more second generation buds subtended in the axils of the fourth to eighth cataphyll of the first generation buds are those destined to produce eventually more or less typical annual shoots a year after the expansion of the first generation buds. These remain very small the first year while they are second generation buds. The small, undeveloped second generation buds and patches of meristematic tissue in the axils of the lowest cataphylls of first generation buds often remain dormant for several years, only to begin renewed activity early some spring.

During the expansion of the winter bud into a shoot in spring, growth and

foliar differentiation are resumed in the second generation buds, which at this time are approximately nine months old. From now on these buds will be termed first generation buds, since they are destined to produce the annual shoot the following year. In a first generation bud cataphyll differentiation continues through the early summer (fig. 14, 15, pl. 18). During the last of July or early in August foliage leaf formation begins. In some of these first generation buds flower organ formation is completed by early October, while in a few cases foliage leaf production is continued during bud expansion the following spring.

The first generation bud increases in size during the winter, due to the enlargement of the foliar organs, and to the slight internodal elongation between the upper cataphylls (fig. 8, pl. 18). During the last of March and in April internodal elongation in the region of the upper cataphylls and foliage leaves and the growth and reflexing of the foliar organs complete the expansion of the bud into the shoot.

Flowering occurs in May. By the last of July the seeds are ripe, or are ripening, after which the aerial portion of the plant usually shrivels.

The foliar cycle of *Paeonia* begins with the germination of the seed and continues from year to year by the complicated bud production just described. Unfortunately, the transition from young seedling to the mature " clump " has not been observed, as the authors have been unable to germinate any seeds or procure young seedlings of *Paeonia*.

Organization and form of cataphylls and foliage leaves

The upper cataphylls, which are often over five centimeters long, are spatulate in form with a vaginate base and a mucronate to obtuse apex (fig. 15–18, pl. 19). In contrast, the lowest cataphylls (i.e., the prophylls) are broadly deltoid in shape, and while often a centimeter in width, are rarely more than three or four millimeters high (fig. 1, 2, pl. 19). As is frequently the case in dicotyledonous buds (cf. Foster, 1929, pl. 46; 1931b, pl. 41), there is a progressive acropetal change in form in the cataphyllary series in *Paeonia,* the middle scales being somewhat ovate or linear in form (fig. 3–14, pl. 19).

Normally, the vaginate portion of all cataphylls in *Paeonia* is tipped by a rudimentary lamina (fig. 1 *a-b* to 18 *a-b,* pl. 19). In only two instances were completely unsegmented cataphylls found (fig. 20, 21). The organization and form of these apparently exceptional organs are quite similar to those of certain of the inner sepals of the flower of *Paeonia* as pictured by Domin (1911, pl. 1, fig. 12 *d*). The structure of the cataphyllary lamina varies according to the relative position of the scale on the shoot. Usually the laminae of the lowest and highest cataphylls are ternately segmented, sometimes consisting of trios of rudimentary leaflets (fig. 1 *a-b* and 12 *a-b* to 17 *a-b,* pl. 19). In the middle series of cataphylls, however, the tiny lamina is often represented only by a single median leaflet (fig. 4 *a-b* to 6 *a-b,* pl. 19). Many irregularities in structure of the cataphyllary lamina occur. Some of

these irregularities are due to the more rapid growth of the lateral leaflets or group of leaflets as compared with the median portion of the blade (fig. 11 *a-b*, 14 *a-b*, and 15 *a-b*, pl. 19). In other cases pronounced asymmetry occurs, due to inequalities in the number of leaflets in the median and lateral portions of the lamina (fig. 17 *a-b*, 18 *a-b*, pl. 19). As in the case of *Aesculus Hippocastanum* L., where comparable irregularities and conditions of asymmetry obtain in the cataphyllary lamina, the factors responsible for the structural variations in the cataphyllary laminae of *Paeonia* are completely obscure at present (cf. Foster, 1929, p. 461; fig. 7 *a*, 7 *b*, pl. 47; fig. 8, pl. 48).

Regardless of form or its position on the shoot, the cataphyll of peony possesses a trilacunar node (fig. 1 *a-b*, pl. 20). The vein system of the prophyll consists of a weakly branched midvein and two ternately branched lateral veins (fig. 10, pl. 20). The venation of the lower cataphylls is very simple. In these the midvein and lateral veins are comparatively little branched (fig. 11, pl. 20). In contrast to the venation of the lower cataphylls, the upper cataphylls have a complex " pseudo-parallel " type of vein system. All references to type of branching, anastomosis, and course of veins in the cataphylls and foliage leaves are purely descriptive. The actual method of formation of the vein system in the organs remains to be investigated. Figure 12 *a* of plate 20 shows the last cataphyll in the winter condition, with much branched lateral veins and a little branched midvein. During bud expansion in the spring intercalary growth in the basal region of the cataphyll causes a prominent elongation of the portion innervated by the three main systems of veins. As a result the reticulately veined region is confined to the upper portion of the adult scale (fig. 12 *b*, pl. 21). In the mature condition the midvein maintains its identity to the tip of the cataphyll, where it divides into three branches, one entering each of the three rudimentary groups of leaflets. The basal cataphylls do not become acrescent; consequently little or no change in the vein system occurs during the expansion of the bud.

The foliage leaves are typically ternately compound, each leaflet being lance-ovate and incised into three to five lanceolate-acuminate lobes; or they are sometimes bi-ternately compound. Often the laminar wings extend well down on the petiolules and petiole, and merge with the petiolar ridges (fig. 4, pl. 20). The foliage leaves vary from eight to thirty centimeters in length, dependent on the vigor of the axis and their relative position on the shoot. In general, the largest and most highly segmented foliage leaf is the one immediately above the uppermost cataphyll. On each aerial shoot there is usually a gradual "transition" from a condition of bi-ternately compound foliage leaf immediately above the highest cataphyll through ternately compound forms with five-lobed, three-lobed, and entire leaflets, followed by simple three-lobed foliage leaves, to organs with simple, entire laminae, as is the case in the uppermost (fig. 4-9, pl. 20). The ventrally channelled petiole, which is thickened at its point of attachment to the stem, is one-fifth to one-half of the length of the foliage leaf.
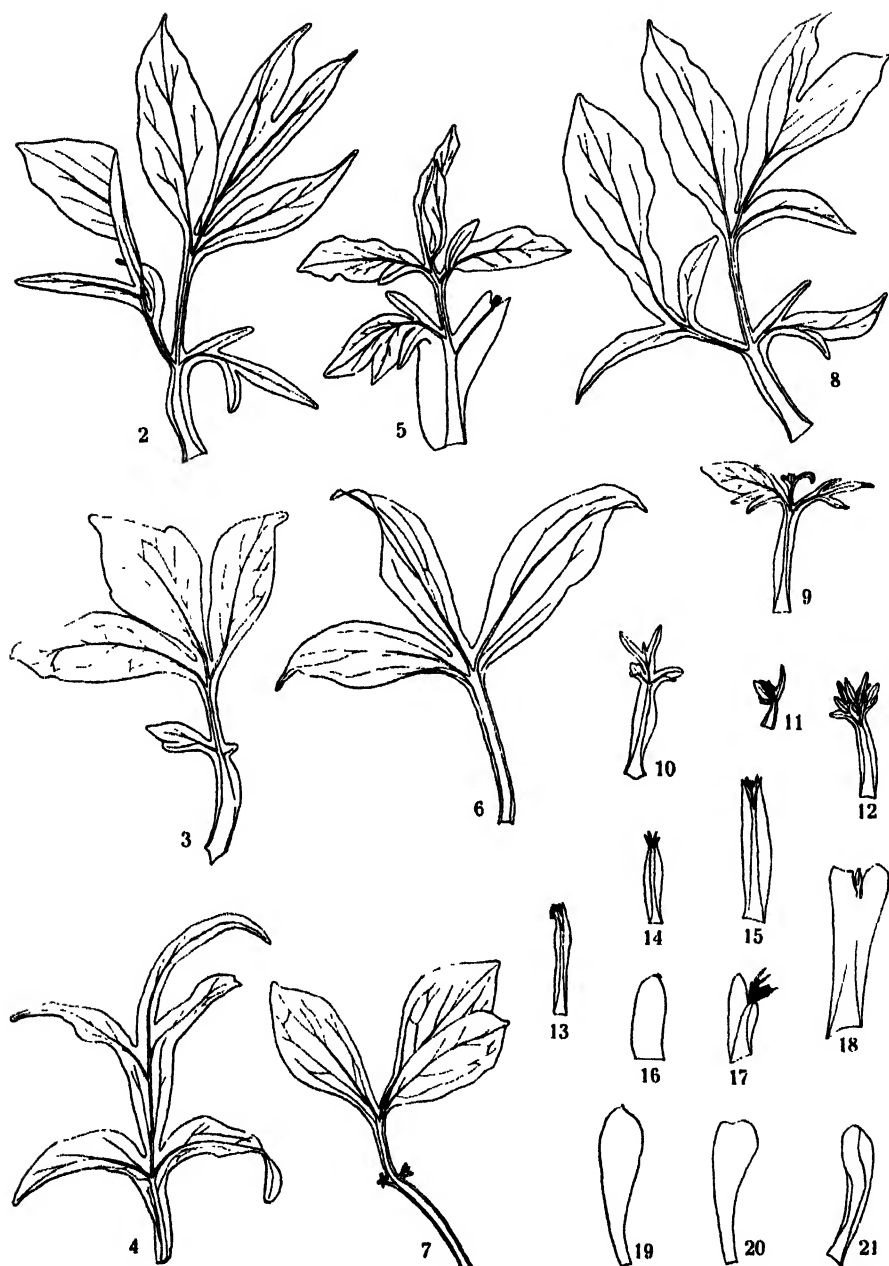
Fig. 2–21. Various types of cataphylls and transitional forms from expanded shoots. Fig. 2–18, transitional forms illustrating the range in degree of development obtaining in the laminar and basal regions. Note the continuity between the blades of the lower leaflets and the basal wings shown in many cases. In figures 2–15, only the main laminar veins are shown. Fig. 19, an uppermost cataphyll with a single apical leaflet. Fig. 20–21, two uppermost cataphylls showing no differentiation into laminar and basal regions. Apparently an unusual condition in *Paeonia*. × 1/2.

As is the case with the cataphylls, the foliage leaf possesses a trilacunar node (fig. 2 *a-b*, pl. 20). The median trace is the first given off. It produces two branches at the level where the two lateral traces enter the petiole. Each of the lateral traces immediately gives off two branches. The outer branches of the lateral veins run up through the ventral petiolar ridges, enter the laminar wings, and diverge as small veins innervating the outer part of the lowest leaflets. Usually just above the leaf base the principal veins anastomose, producing a wide median dorsal bundle in the petiole. At the point where the petiolules join the petiole, the median dorsal bundle divides into three branches. In a few cases, however, three broad bundles extend through the petiole to the point just below the attachment of the petiolules (fig. 3 *a-c*, pl. 20), where they unite only to divide again into three cords. In either case, each group of leaflets of a bi-ternately compound, or each leaflet of a ternately compound foliage leaf, receives one main dorsal bundle (fig. 3 *d-e*, pl. 20).

While the last cataphyll on each tiller is ordinarily followed by a typical foliage leaf, in some cases foliar organs are found whose construction and position on the axis seems to be transitional between cataphyll and foliage leaf. A number of these organs are shown in figures 2–18.

These transitional forms usually seem in each case to be the uppermost cataphyll. Variations in the upper cataphyll and transitional forms of various tillers range from organs possessing a sheathing portion with no indication of a lamina (fig. 20, 21), through those in which the laminae of the cataphylls are in various stages of expansion, and the sheathing portion in various degrees of transition from the typical sheathing element of the normal cataphyll to the petiolar form of the normal foliage leaf (fig. 2–18).

In cases where the lamina is much reduced, the form and venation of the petiolar portion are very similar to those of the sheathing part of the cataphyll (fig. 5, 10–18); while in cases where the lamina is broadly expanded and highly segmented, the petiolar portion is much thickened and is very similar to the petiole of a normal foliage leaf, although usually somewhat broader (fig. 2–4, 6–9). Ordinarily, there is little or no differentiation between petiole and leaf base in transitional forms.

Often the winged portion of the petiole is continuous, in transitional forms, with the lamina of the lowest leaflet or lobe. Reference to figures 2–6 and 8–10 will show the prevalence of the continuity between the lamina and the sheathing portion of the petiole in transitional forms. In such cases the vein system of the lateral portion of the petiole and the lower half of the adjoined lamina is continuous.

Ontogeny of cataphylls and foliage leaves

Very early in ontogeny important external differences appear between the cataphylls and the foliage leaves. For purposes of clearness the development of each organ will be discussed separately.

Regardless of its adult form, the primordium of a cataphyll first appears

as a crescentic swelling at the growing point (fig. 14, 15, pl. 18; fig. 1, 2, pl. 21). Further development at first involves a rapid increase in size of the scale initial; in particular, its basal width tends to increase at a faster rate than its length.

Differentiation into lamina and basal portion occurs, when the cataphyllary primordia are from one-half to one millimeter in width and from one-half to two-thirds of a millimeter in length, by the appearance of a pair of leaflet initials that appear as two ventri-lateral swellings (fig. 3, pl. 21). These two initials, with the apical part of the primordium, produce the three leaflets typical of the cataphyllary lamina. While this is a frequent condition, many variations occur. In some cataphylls, usually in those of the middle series on the axis, laterals do not appear (fig. 4 a-b to 6 a-b, pl. 19). In other cataphylls a pair of lateral papillae appear at the base of each of the three original initials, resulting in three ternate groups of rudimentary leaflets (fig. 5, pl. 21).

Growth in the apical region of the cataphyll is rather sluggish, resulting in a lamina that rarely exceeds one millimeter in length except in the so-called "transitional forms." During the winter and during the expansion of the bud rudimentary laminar wings are produced by marginal growth of the leaflet primordia.

In contrast to the sluggish growth of the lamina, rapid growth in the basal region of the primordium is the dominant factor determining the shapes of the cataphylls.

The lowest cataphylls—i.e., the prophylls—in common with the prophylls of most dicotyledons are lateral to the median plane of symmetry of the axillant leaf (text fig. 1; fig. 5, 6, 12, 13, pl. 18). Further growth in the prophylls, after their early appearance as crescent-shaped primordia, is predominantly lateral and accompanies the increasing girth of the bud. As a result of their early maturation the prophylls of expanding buds are usually found ruptured.

In the upper series of cataphylls expansion in length and width is about equal during early development. Later, lateral extension as a result of active marginal growth is most rapid near the apical end, resulting in the characteristic apical wings and the constricted bases of the cataphylls (fig. 12 a, pl. 20; fig. 6–8, pl. 21). This type of growth produces the obovate form typical of these organs in the winter bud. The upper cataphylls are not torn by the increasing girth of the bud, but increase in basal width, as the axis becomes larger. Further increase in size in the upper cataphylls, following the embryonic period of development, apparently is brought about during the winter enlargement and the expansion of the bud in spring by intercalary growth principally localized in the basal region. This basal growth results in the obovate or spatulate form of the adult upper cataphylls (fig. 12 b, pl. 20; text fig. 23).

The development of the foliage leaf differs from that of the cataphyll in the earliest observed stage of growth, although at certain early periods of their

ontogeny the foliage leaf and cataphyll primordia resemble each other markedly (pl. 21).

In contrast to the crescent-shaped primordia of the cataphylls, the foliage-leaf initial first appears as a papillate swelling at the growing point (fig. 9,

Fig. 22 and 23. Dorsal aspects, respectively, of the first foliage leaf and the last cataphyll of an expanding bud in spring. The foliage leaf shows a clear demarcation into leaf base, petiole, and lamina; veins are rapidly developing in the latter (cf. fig. 9–16, pl. 21, for earlier developmental stages). The cataphyll consists of a prominent vaginate portion tipped by a rudimentary lamina. This represents the final stage of cataphyll development (cf. fig. 1–8, pl. 21, for earlier developmental stages.)   × 5.

pl. 21). Following this developmental stage, the papillate foliage-leaf initial rapidly increases in basal width, height, and thickness. When approximately two hundred micra in width and three hundred micra long, differentiation into

lamina and basal region occurs. These dimensions, although only of relative value, clearly show that differentiation into laminar and basal regions occurs earlier in the foliage leaf than in the cataphyll (cf. p. 375).

The mode of development of the lamina is related to the position of the foliage leaf on the axis. In the simplest case, as represented by the uppermost foliage leaves, the apex of the primordium develops into a small, undivided blade (fig. 9, pl. 20). Lamina formation, however, in the lower, highly segmented foliage leaves is more complex. The primordium of such organs early develops, near its apex, two ventri-lateral papillae (fig. 10–12, pl. 21). The latter, together with the apex of the initial, represent the three primary lamina segments. In certain cases further elaboration of the lamina ceases at this stage, resulting in the trilobate type of blade (fig. 7, 8, pl. 20). The bi- or tri-ternately compound lamina, characteristic of the lowermost foliage leaves, is formed as follows. Each primary lamina segment develops a pair of ventral swellings near its base. These protuberances increase rapidly in size, soon acquiring a form similar to the segment from which they have developed (fig. 13, pl. 21). If no further elaboration of the lamina occurs, these three groups of leaflet primordia lead directly to the bi-ternate type of lamina (fig. 5, pl. 20). Very frequently, however, the structure of the lamina becomes more complex as the result of the formation of additional leaflet primordia. The latter arise most commonly from the central trio of main leaflets (fig. 14, pl. 21). Often the amount of segmentation of the median and lateral groups of leaflets varies considerably, and the leaflets and lobes in each of the three leaflet groups are sometimes markedly asymmetrical (fig. 4, 5, pl. 20).

The leaflet primordia grow at first equally around their adaxial margins, but by the time the foliage leaf initial is a millimeter long, this type of growth ceases at the bases of the leaflets. Lateral expansion around the extremities of the leaflets continues without noticeable interruption until the veins become differentiated, after which continued growth is shown principally by the general expansion of the entire leaflet lamina. The veins first appear externally noticeable in the median leaflet (fig. 16, pl. 21) when it is between two and three millimeters in length. Later they appear in the lateral leaflets and lobes (fig. 22).

During and accompanying the appearance and growth of the laminae, rapid lateral extension and growth in thickness occur, in addition to some growth in length, in the basal region of the foliage-leaf primordium. The dorsal thickening produces a keel down the center of the primordium, while the lateral growth results in the formation of narrow, wing-like extensions (fig. 12–16, pl. 21; text fig. 22).

Intercalary extension and thickening at the bases of the three groups of leaflets result in the formation of the petiolules, while similar growth below the point of union of the petiolules, mostly in the narrow region above the broad base, produces the petiole (fig. 15, 16, pl. 21). Further development

38

and maturation of the petiolules and petiole occur simultaneously with the expansion of the leaflets during the opening of the bud in the spring (fig. 22).

In view of the sporadic occurrence of the transitional forms, it has not been possible to investigate their ontogeny with accuracy. The evidence at hand, however, suggests that in the development of these organs laminar formation proceeds more actively than in the cataphylls proper, although marginal growth in the basal region is still strongly emphasized. Reference to figures 2–18 will indicate the polymorphism which may result according to the relative dominance of growth processes characteristic of the cataphyll or the foliage leaf.

DISCUSSION

From a morphological standpoint it is of interest that both cataphyll and foliage leaf in *Paeonia* agree in the possession of an apical lamina. Of further importance is the fact that the cataphyllary lamina, although minute and relatively undeveloped, is usually ternately segmented, a feature corresponding exactly to the basic architecture of the foliage-leaf lamina. Furthermore, the outer leaflets of the cataphyll are usually continuous with the margins of the vaginate basal portion of the scale in a manner comparable to the relationship between the laminar wings, petiolules, and petiole of the foliage leaf. In view of these facts, the morphological descriptions of the cataphyll of *Paeonia* by previous investigators appear surprising. DeCandolle (1827, pl. 21, fig. 1, 2, 3), for example, depicts the cataphylls as unsegmented structures, and Clos (1856, p. 684), although attempting to homologate the scale with the sheath portion of the foliage leaf, fails to mention the apical leaflets. Glück (1919, p. 167) simply describes the cataphylls of *Paeonia* as oval, unsegmented structures. These statements from the literature must rest upon observational error, for in the very large number of cataphylls examined during the present study, only two were found to be unsegmented in character (cf. text-fig. 20, 21).

Thus, in general organization the cataphylls of *Paeonia* are fundamentally similar to the bud scales of *Acer pseudoplatanus* L. (Schüepp, 1929), and *Aesculus Hippocastanum* L. (Foster, 1929) in that they consist of a prominent vaginate basal portion surmounted by a rudimentary lamina. When this type of organization is compared ontogenetically with that of the foliage leaf, several important facts emerge.

First of all, the similarity in organization and mode of early development of the lamina of cataphyll and foliage leaf is suggestive of a very close morphological relationship between these organs. That is, both scale and foliage leaf primordia exhibit a similar " polarity " or " working plan " which is expressed by segmentation into lamina and basal regions (fig. 3, 11, pl. 21). (Cf. Schüepp's [1929, p. 799–800] theoretical analysis of scale and foliage-leaf development in *Acer*.) This expression of " polarity " during the ontogeny of the two organs, however, is not indicative of a " transformation " process (in Goebel's [1884, p. 251] sense), for the *time* of appearance

of the lamina primordium and its *rate* of development in the scale are quite unlike those in the foliage leaf. Indeed, the evidence at hand suggests that, as in the case of *Acer* and *Aesculus,* the cataphyllary lamina arises *after* certain " determinative " processes, associated with scale development, have appeared in the primordium. Careful experiments, however, of the general type performed by Schüepp (1929) on *Acer* are required to test the validity of this assumption.

Profound differences in form and organization appear, however, when the sublaminar regions of the cataphyll and foliage leaf are compared. The basal portion of the scale is sheath-like in form and texture, and in its dorsiventral construction is quite unlike the corresponding thickened petiolar-leaf base region of the foliage leaf (fig. 22, 23). These striking differences between cataphyll and foliage leaf arise early in ontogeny and can be analyzed as follows. Characteristic growth processes in the foliage leaf at an early stage consist in (*a*) the rapid formation of a lamina from the epipodium and (*b*) the demarcation in the basal region of a thickened mesopodium and a narrowly winged hypopodium. The terms epipodium, mesopodium, and hypopodium are used in Bower's (1916) sense purely as convenient terms descriptive of topography. During bud expansion the lamina experiences rapid enlargement and maturation, while the mesopodium elongates, forming the adult petiole. The hypopodial region, except in transitional organs, is not noticeably broadened at maturity. In contrast, the early ontogeny of the cataphyll is distinguished by (*a*) the relatively late appearance and sluggish development of the lamina and (*b*) the early dominance of growth in the basal region of the primordium. As the result of active marginal growth and the failure of noticeable increase in radial thickness, the basal region of the scale soon becomes vaginate in form and shows no clear distinction into hypopodial and mesopodial regions. As in the case of *Aesculus,* this basal region of the scale owes its characteristic form to the early formation of massive wing-like extensions. Although the exact mode of development of the vascular system of the scale requires investigation, it appears likely that its distinctive features are related to marginal growth. Further development of the scale during bud expansion consists mainly in the rapid enlargement of the sheathing portion, accompanied by the formation of rudimentary blades on the leaflet primordia.

It is apparent from the ontogenetic analysis just given that the divergence in form between the cataphyll and foliage leaf in *Paeonia* is due largely to differences in the type of growth occurring in the basal region of their respective primordia (pl. 21). An investigation of these specific growth differences from a histogenetic and experimental standpoint may shed light upon the exact point at which scale or foliage-leaf " determination " occurs. The results of the present study simply indicate that while a foliar primordium in peony may be " labile " at a very early stage, definite and specific growth

processes quickly appear which permit accurate judgment of its ultimate destiny.

The following scheme (fig. 24) illustrates two possible ways of viewing the problem of foliar morphology in *Paeonia*. One might compare the four types of adult foliar organs represented as end products in the corresponding
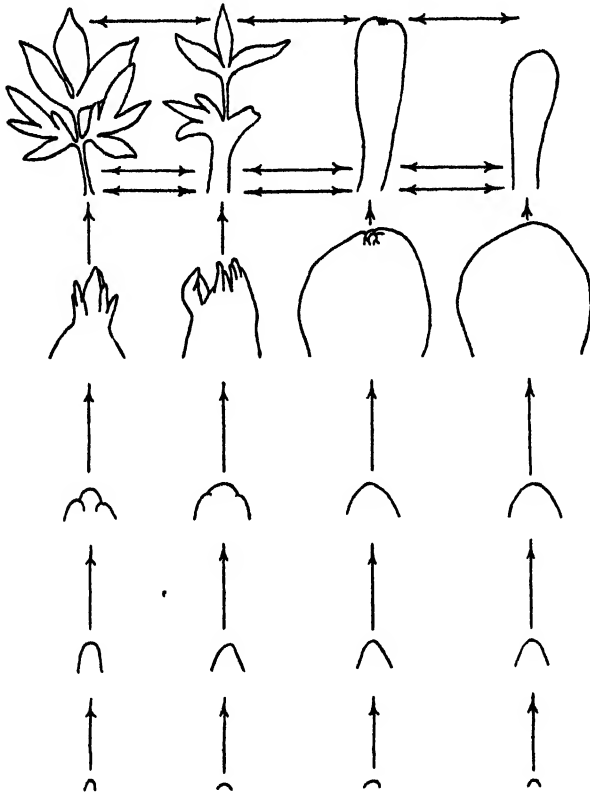
Fig. 24. Diagram illustrating two possible ways of interpreting the problem of foliar morphology in *Paeonia*. From a phylogenetic viewpoint one might regard either the foliage leaf, shown at the upper left of the diagram, as the starting point from which progressively the transitional form, the typical cataphyll, and the unsegmented cataphyll have arisen, or the unsegmented cataphyll might be taken as the starting point (illustrated by the series of horizontal arrows). From an ontogenetic viewpoint one might interpret the four types of organs as end products of specific ontogenies, the nature of which are available for observation and experimentation (illustrated by the four vertical series of arrows).

ontogenetic series—viz., the foliage leaf, the transitional form, the typical cataphyll, and the unsegmented cataphyll. In comparing such end products from a phylogenetic viewpoint, one would need to begin logically either with the foliage leaf or with the unsegmented cataphyll. Both possibilities have been adopted by morphologists for similar cases in the past, but in view of

our ignorance of the details of foliar evolution in the angiosperms, the results of such speculations have not carried us far (cf. Foster, 1931a). An alternative viewpoint, for the present at least, consists in regarding these variously constructed foliar organs as end points of specific ontogenies. Reference to figure 24 will indicate that the various types of organs originate from an apparently simple mass of meristem—i.e., the foliar primordium. As has been pointed out previously, the ultimate destiny of a given foliar primordium depends upon certain definite and specific growth processes which appear early in ontogeny. An effort has been made to analyze some of these growth processes in this paper. Further study of the problem from this standpoint may result in a better understanding of the factors controlling foliar development in *Paeonia*, and hence may contribute to our still meagre knowledge of the process of organ determination in plants.

SUMMARY

1. The present investigation deals with the organization and development of cataphylls and foliage leaves in *Paeonia officinalis* L. hort. var. Zoe Callot.

2. The shoot system is a sympodium, as a result of the origin of the annual shoots from subterranean axillary buds found near the base of the previous year's tillers.

3. Buds destined to form the annual shoots are initiated at least twenty months before their expansion.

4. The foliar composition of the annual shoot is variable, ranging from nine to twenty-one cataphylls followed by six to fifteen foliage leaves. All shoots are potentially fertile, but in many cases the floral organs are abortive.

5. The adult foliage leaf consists of a well-developed lamina, a thickened petiole, and a leaf base. The laminae of successive foliage leaves show a progressive reduction from the bi-ternate organization of the lowest organs through ternately compound and trilobate forms to a simple undivided blade in the uppermost organs.

6. The adult cataphyll consists of a prominent vaginate portion usually tipped by a rudimentary lamina. Generally the laminae of the lower and uppermost cataphylls are ternately segmented, while in the middle scales of the bud the blade is represented by a single median leaflet.

7. The node of both cataphyll and foliage leaf is trilacunar. The vascular bundles are arranged in a crescentic series in the leaf-base and petiole of the foliage leaf, while the venation of the sheathing portion of the cataphyll is " pseudo-parallel " in type.

8. Cataphyllary primordia appear as crescentic swellings at the growing point and differentiate relatively late into a rudimentary sluggishly growing lamina and a rapidly enlarging basal region. Active marginal growth in the basal region produces the characteristic vaginate form of the adult cataphyll.

9. Foliage-leaf primordia appear as papillate swellings at the growing point and differentiate relatively early into a rapidly growing lamina and a

basal region. The latter is soon demarcated into a thickened mesopodium (which forms the petiole) and a narrowly winged hypopodium (which forms the leaf base).

10. The primordia of cataphylls and foliage leaves in *Paeonia* exhibit a similar " polarity " which is expressed by segmentation into lamina and basal regions. Differences in the rate and type of growth in these two regions are responsible for the divergence in form between the adult cataphyll and foliage leaf.

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EXPLANATION OF PLATES

PLATE 18

The structure and periodic development of the bud in *Paeonia*.

Fig. 1. A portion of the subterranean root and shoot system of peony as it appears in early spring. Note the adventitious roots and the two large buds; the latter form the annual shoots or tillers. × 1/4.

Fig. 2. A partially developed annual shoot which has arisen from a subterranean winter bud. Note the accrescent character of the upper cataphylls, the cluster of expanding foliage leaves, and the apical group of flower buds. × 1/4.

Fig. 3. The aerial portion of an annual shoot just prior to flowering. Note the large terminal flower bud and the two branches developed from buds in the axils of the upper cataphylls. × 1/4.

Fig. 4. Portion of the underground shoot system during June. At the left is the basal region of an annual shoot with well-developed first generation buds. This shoot has arisen from a tiller of the previous year, shown at the right. × 1/2.

Fig. 5. Abaxial view of a first generation bud in June. Note the unequal size of the two laterally placed prophylls. × 6.

Fig. 6. Abaxial view of a first generation bud in August. Note the laminar primordia of the prophylls and the outer exposed cataphylls. × 10.

Fig. 7. A first generation bud during the winter with all but the last cataphyll removed. Note the small second generation buds which have arisen in the axils of the uppermost cataphylls of this first generation bud during the latter part of the preceding summer. The cluster of small foliage leaves is partially embraced by the last cataphyll. × 4/3.

Fig. 8. An enlarged view of a first generation bud during the winter with all cataphylls removed. Note that the uppermost of the three large second generation buds is scaleless. Frequently certain of these upper second generation buds form branches during the expansion of the first generation bud in spring, as shown in figure 3. × 3/2.

Fig. 9. Top view of a very young developmental stage of a second generation bud which appears as an oval mass of meristem in the axil of a cataphyll of the first generation bud during June. This second generation bud is destined, about twenty months later, to produce an annual shoot. × 40.

Fig. 10. Top view of a first generation bud, illustrating an older developmental stage of a second generation bud. The latter, shown at the lower right of the figure, has formed the primordium of one prophyll. × 24.

Fig. 11. Lateral view of a first generation bud showing a slightly older developmental stage of a second generation bud. As in figures 9 and 10, the subtending scale of this bud has been removed. × 24.

Fig. 12. An enlarged lateral view of the second generation bud depicted in figure 11. The rapidly developing prophyll of this bud is shown at the left of the somewhat flattened growing point. × 40.

Fig. 13. Lateral view of a second generation bud during October. Note the increase in size which has occurred. The two prophylls are unequal in size. × 24.

Fig. 14, 15. Formation of cataphyll primordia at the growing point of a first generation bud in June. At this general period, second generation buds are appearing in the axils of the lower cataphylls of the first generation bud. × 24.

PLATE 19

Organization and form of a complete series of adult cataphylls from a single annual shoot.

Fig. 1–18, successive cataphylls of the annual shoot illustrating the rudimentary character of the apical laminae and the progressive change in form of the vaginate portion of the cataphyll. × 1.

Fig. 1a-1b, 2a-2b, etc., enlarged dorsal and ventral aspects, respectively, of the laminae of the cataphylls shown in figures 1-18. Further explanation in the text. × 12.

PLATE 20

Organization, nodal anatomy and venation of cataphyll and foliage leaf.

Fig. 1a-1b. Transverse sections, at successive levels, illustrating the trilacunar node of the cataphyll. × 6.

Fig. 2a-2b. Transverse sections, at successive levels, illustrating the trilacunar node of the foliage leaf. × 6.

Fig. 3a-3d. Transverse sections, at successive levels, through the leaf base and petiole of the foliage leaf, illustrating the venation of these regions. Fig. 3a and 3b, sections through the leaf base. Fig. 3c and 3d, sections through the petiole. Fig. 3c, transverse section through the bases of the three main petiolules. × 2.

Fig. 4-9. A representative series of foliage leaves illustrating the progressive reduction in size and complexity obtaining at successively higher nodes of the shoot. Only the main veins of the laminae are shown. × 1/2.

Fig. 10. Organization and venation of the adult prophyll. × 6.

Fig. 11. Organization and venation of a lower cataphyll. × 6.

Fig. 12a and 12b. Form and venation of the innermost bud scale. Fig. 12a, venation of this scale in the winter bud. Fig. 12b, venation of this scale at maturity, after basal elongation has been completed. × 3.

PLATE 21

Fig. 1-8. Early developmental history of the cataphyll. Fig. 1-3, ventral aspects of early stages in development of the upper cataphylls. Fig. 4-8, dorsal aspects of successive developmental stages of the last cataphyll of the bud. Fig. 1, an unsegmented primordium. × 28. Fig. 2, a slightly older primordium. Note that while active marginal growth is occurring, the laminar primordium has not yet appeared. × 28. Fig. 3, a primordium at a much later stage showing differentiation into a small trilobate lamina and a rapidly growing vaginate portion. × 28. Fig. 4, a stage following that depicted in Fig. 3, showing that increase in size is due to the dominant growth of the vaginate portion of the primordium. × 28. Fig. 5, a later stage illustrating the relatively slow segmentation of the cataphyllary lamina which now consists of three ternate groups of leaflet primordia. Note that wing-formation is most active some distance above the base of the primordium. × 28. Fig. 6 and 7, slightly older developmental stages, illustrating the sluggish growth of the laminar primordium and the continued rapid growth of the wings in the vaginate portion of the initial. × 15. Fig. 8, a characteristic stage which occurs in the winter bud. Note the relatively small size of the ternately segmented laminar primordium as compared with the large, obovate, vaginate portion of the cataphyll. × 7.

Fig. 9-16. Early developmental history of the foliage leaf. All figures illustrate primordia at approximately the same age and scale of magnification as shown in the corresponding series (fig. 1-8) depicting cataphyll development. Fig. 9-11, ventral aspects of young developmental stages of the foliage leaf. Fig. 12-16, dorsal aspects of successive developmental stages of the first foliage leaf of the bud. Fig. 9, an unsegmented primordium. × 28. Fig. 10, a later stage illustrating the early segmentation of a foliage-leaf initial into laminar primordium and basal region. Compare with figure 2. × 28. Fig. 11, a slightly older developmental stage illustrating the rapid development of the three primary laminar lobes. Compare with the corresponding stage in cataphyll development, figure 3. × 28. Fig. 12, a slightly older stage, showing the relatively slow growth occurring in the basal region of the primordium. × 28. Fig. 13.
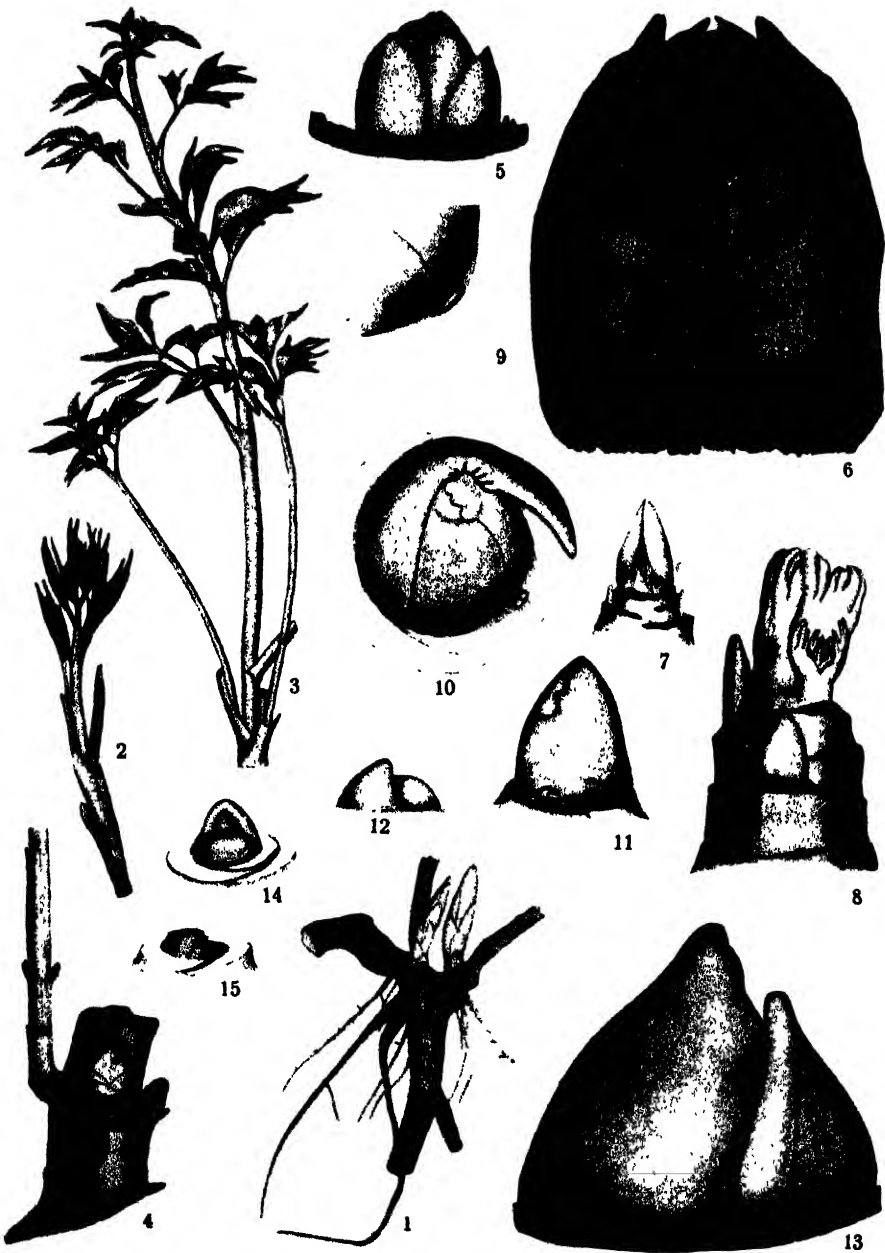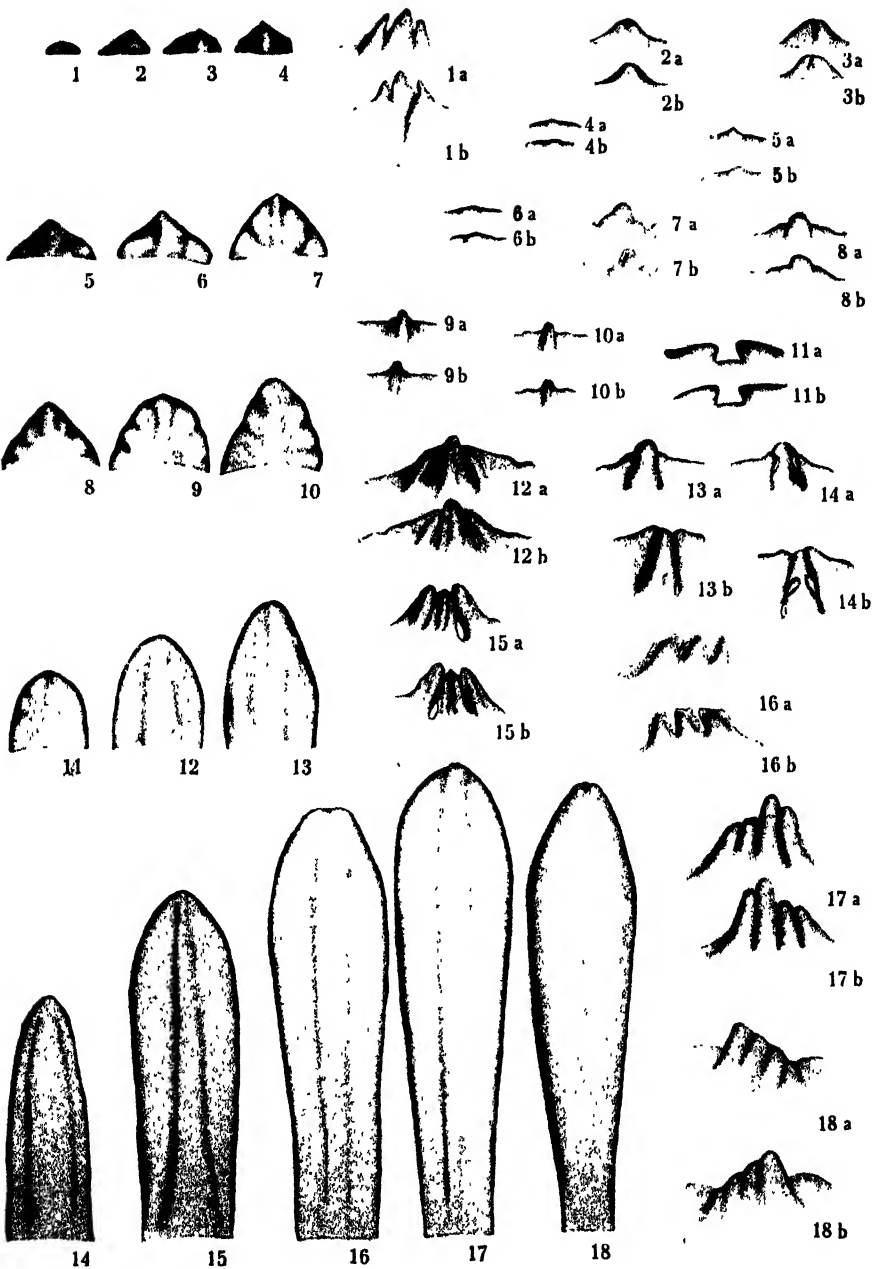
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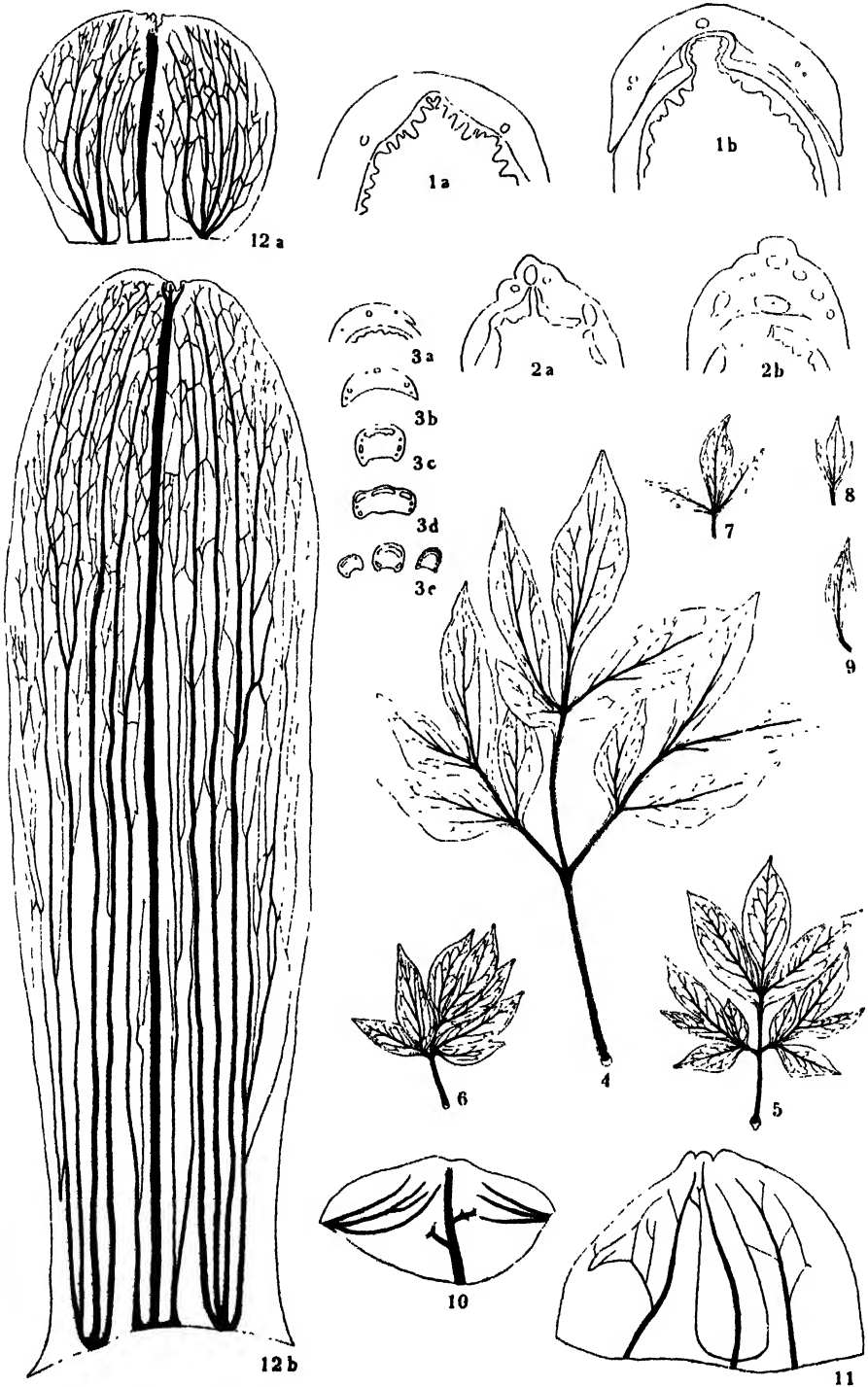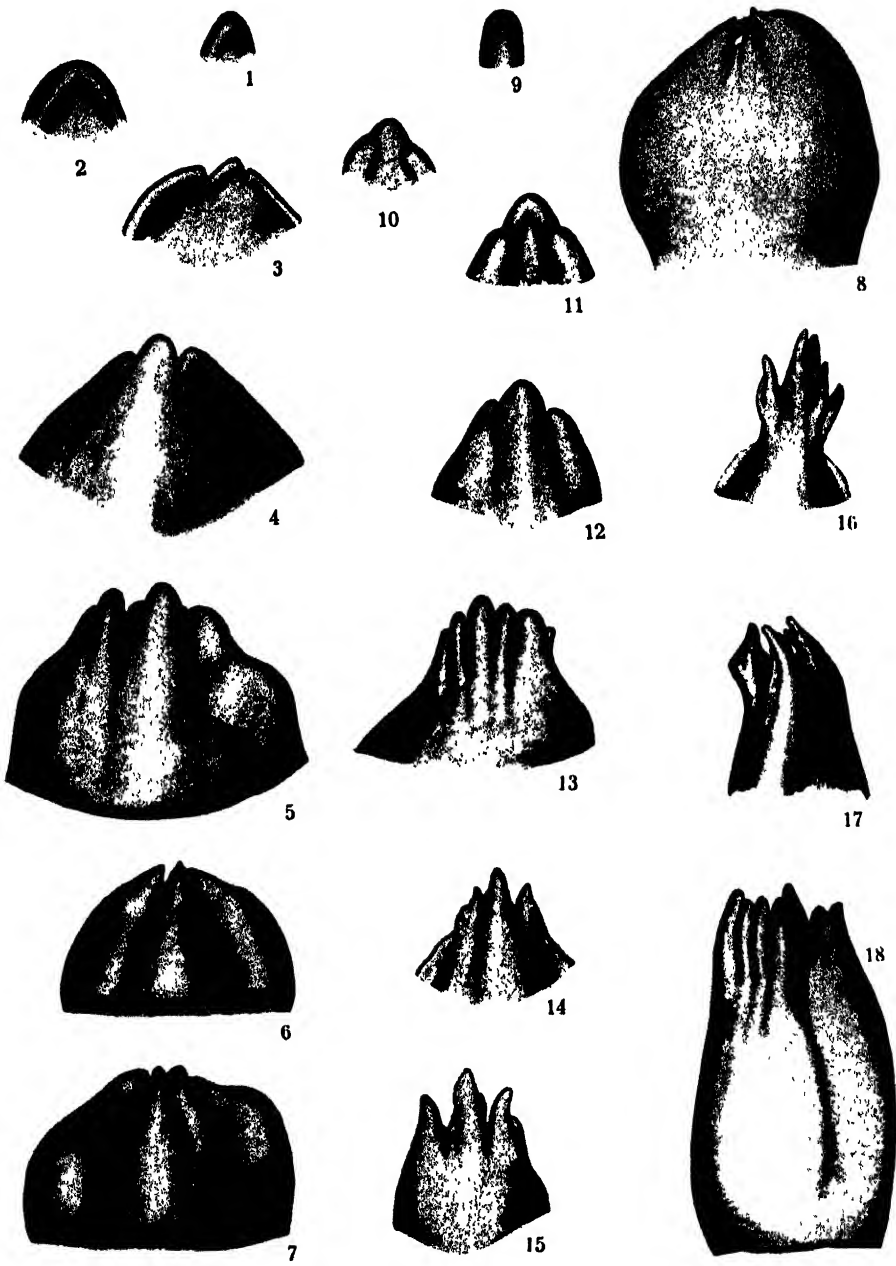
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a later stage, illustrating the further segmentation of the laminar primordium. Compare with figure 5. × 28. Fig. 14 and 15, much older developmental stages, emphasizing the rapid and comparatively elaborate differentiation of the laminar primordium and the slow increase in width of the sublaminar region. Compare with figures 6 and 7. × 15. Fig. 16, a characteristic stage which occurs in the winter bud. At this stage, the foliage leaf consists of a well-developed lamina, a short, thickened mesopodium, and a narrowly winged hypopodium. Note that a midrib and lateral veins are already partially differentiated in the terminal leaflet. Compare with the rudimentary character of the cataphyllary lamina at a corresponding developmental period shown in figure 8. × 7.

Fig. 17. Dorsal aspect of a transitional form dissected from a winter bud. This organ is depicted at approximately the same scale of magnification as figures 8 and 16. Note that the laminar and basal region of this organ are intermediate in character as compared with the "typical" cataphyll and foliage leaf. × 7.

Fig. 18. Abaxial view of an upper second generation bud. The prophyll at the right has a typical cataphyllary organization, while the second organ, shown at the left, is a small foliage leaf. This figure emphasizes the striking contrast between the organization typical of cataphyll and foliage leaf. × 9.

THE GENETICS AND CYTOLOGY OF TWO FIFTEEN–CHROMOSOME MUTANTS FROM THE HAPLOID OF *OENOTHERA FRANCISCANA* [1]

JOHN EDWARD ANDERSON

INTRODUCTION

This report presents the results of cytological and genetical studies on two trisomic mutations occurring in *Oenothera*. It is hoped that the study will throw some light on the problem of the synaptic behavior in *Oenothera* and on the cause or causes of sterility of the particular form so commonly exhibited by chromosomal variants of the $2n + 1$ type. Inasmuch as a detailed statement of the pedigree and breeding data for each plant appears in tables 1 and 2, it will suffice here to make only a brief statement of the origin of the two variants.

Oenothera franciscana Bartlett, a wild species carried through fifteen generations by Davis, gave rise in 1923 to its first variant, a haploid called Pointed Tips (Davis and Kulkarni, 1930). Selfed seed of this haploid produced in 1924 (culture 24.25, table 1) a progeny of 329 *franciscana*, and in addition two more plants of the haploid. Seed from the 1924 haploids selfed, when planted in 1926 (culture 26.26, table 1), gave 55 *franciscana* and one plant dwarf like the haploid, but spreading and symmetrical in habit, quite rigid, and with broader leaves. This mutant was named Bushy Dwarf (fig. 1). In 1927, from seed of selfed haploids of 1926 (culture 27.41, table 2), there appeared, in addition to 107 of *franciscana* and two haploids (Pointed Tips), 13 dwarfs having long narrow leaves, a dense, low habit, and buds which were red and very long. Because of the character of the buds, this mutant was called Red Elongate (fig. 2). As shown in tables 1 and 2, the sports have arisen frequently in later cultures.

Since the two 15-chromosome mutants arose from seed of the 7-chromosome haploid selfed, the origin of the 8-chromosome gamete which must unite with the normal 7-chromosome gamete becomes a matter of interest. Davis and Kulkarni (1930) have shown that the small amount of fertile pollen developed by the haploid of *franciscana* results from the suppression of the heterotypic division to give a restitution nucleus which then goes through the homeotypic division to produce two pollen grains. Occasional

non-disjunction in the homeotypic division (probably in the embryo sac) would give the 8-chromosome gamete necessary for the production of a 15-chromosome zygote. There may be seven possible types of 15-chromosome zygotes, since any of the 7 chromosomes of the *franciscana* set might be involved in non-disjunction. Such a group of seven trisomic forms would correspond to the twelve primaries established for *Datura* by Blakeslee and his co-workers.

Red Elongate and Bushy Dwarf differ from the parent, and in addition are distinctly unlike one another. Mutations of this type are known from

Fig. 1. Bushy Dwarf, 15-chromosome primary of *Oenothera franciscana.*

Datura Stramonium, where they have been studied in detail in the forms Globe and Poinsettia (Blakeslee, 1921; Blakeslee and Farnham, 1923). Clausen and Goodspeed (1924) in *Nicotiana* have found the sport Enlarged Flower to be of this character. Other forms are the Matthiola mutations of Frost and Mann (1924) and several sports in *Lycopersicum* (Lesley, 1928). In *Oenothera* trisomic sports are well known. Gates (1923), reviewing the literature of such trisomic forms, lists thirty-seven as having been reported from the time when the first chromosome counts of this kind were made in

1908 and 1909 for the *lata* and *albida* of de Vries. Håkansson (1930) has added to the list.

Red Elongate and Bushy Dwarf are non-chain-forming, since all of the chromosomes pair except the three that constitute the trisome and which for this reason are of particular interest. Because of the work of Weier (1930) on *Oenothera Hookeri,* in which parasynapsis was reported, it is to be expected that the same mode of synaptic behavior will be present in the progeny of such a closely related species as *Oenothera franciscana.*

That *Oenothera* exhibits telosynaptic behavior has been the contention of the majority of investigators. Gates (1907, 1909), Davis (1910, 1911), Geerts (1909, 1911), Cleland (1922–1929), Håkansson (1926, 1928), Sheffield (1927, 1929), Sinoto (1927), Illick (1929), and Kulkarni (1929) have

Fig. 2. Red Elongate, 15-chromosome primary of *Oenothera franciscana*

been the foremost supporters of the telosynaptic interpretation. The list of the workers who hold to the parasynaptic interpretation includes Stomps (1910), Boedyn (1924, 1925, 1926), Schwemmle (1926), Lewitzky (1927), Kihara (1927), Darlington (1929, 1931), Weier (1930), Leliveld (1931), and Catcheside (1931a). Due to the work of Weier (1931), Darlington (1931), and Emerson (1931b), the opinions of *Oenothera* cytologists have probably in large measure shifted to the interpretation of parasynapsis. Gates and Goodwin (1931) have reported parasynapsis in *Oenothera purpurata* and *O. blandina* with seven pairs of chromosomes, and Catcheside (1931b) for a derivative from *Oenothera Lamarckiana* with a ring of 6 chromosomes and 4 pairs. Wisniewska (1932) gives evidence for parasynapsis in *Oenothera biennis* and in a form from *O. Hookeri* with a ring of 4 chromosomes and 5 pairs.

40

MATERIAL AND METHODS

The origin of Bushy Dwarf and Red Elongate is given in the Introduction, and tables 1 and 2 present in detail the breeding data for these mutations through later generations, while tables 3 and 4 give the results from back crosses of Bushy Dwarf and Red Elongate to the parent type *franciscana*.

TABLE I. *Pedigree of Bushy Dwarf*

Culture	Parents	% germination	O. franciscana	Bushy Dwarf	Red Elongate	Pointed Tips	Misc. Dwarf	New types	Died	Total
24.25 (Davis)	23.21–165 (Pointed Tips) (Haploid)	94	329	0	0	2	1	0	29	361
26.26 (Davis)	24.25–332 (Pointed Tips) (Haploid)	68.2	55	1	0	0	2	0	2	60
27.43 (Davis)	26.26–40 (Bushy Dwarf) (2n + 1)	53.4	135	21	1	0	1	0	6	164
28.36 (Davis)	27.43–57 (Bushy Dwarf) (2n + 1)	26.2	63	8	0	0	0	0	8	79
29.44 (Davis)	27.43–57 (Bushy Dwarf) (2n + 1)	53.6	186	32	0	0	0	0	12	230
30.35 (Davis)	29.44–215 (Bushy Dwarf) (2n + 1)	81.1	232	26	0	0	0	0	12	270
31.41 (Anderson)	30.35–110 (Bushy Dwarf) (2n + 1)	78.8	177	21	0	2	0	1	42	243
31.42 (Anderson)	30.35–112 (Bushy Dwarf) (2n + 1)	76.2	187	22	0	0	0	2	20	231
32.17 (Anderson)	31.42–33 (Bushy Dwarf) (2n + 1)	70.9	51	10	0	0	0	0	1	62
32.18 (Anderson)	31.42–199 (Bushy Dwarf) (2n + 1)	62.07	18	4	0	0	0	0	0	22
32.19 (Anderson)	31.42–209 (Bushy Dwarf) (2n + 1)	86.2	89	25	0	0	0	2	1	117
32.20 (Anderson)	31.41–61 (Bushy Dwarf) (2n + 1)	78.4	106	40	1	0	0	0	3	150
32.21 (Anderson)	31.46–305 (Bushy Dwarf) (2n + 1)	64.1	65	1	0	0	0	0	0	66
Total			1693	211	2	4	4	5	135	2055

TABLE 2. *Pedigree of Red Elongate*

Culture	Parents	% germination	*O. franciscana*	Red Elongate	Bushy Dwarf	Pointed Tips	Misc. Dwarf	New types	Died	Total
24.25 (Davis)	23.21–165 (Pointed Tips) (Haploid)	94	329	0	0	2	1	0	29	361
26.25 (Davis)	24.25–248 (Pointed Tips) (Haploid)	74	29	0	0	3	0	0	5	37
27.41 (Davis)	26.25–13 (Pointed Tips) (Haploid)	75	107	13	0	2	9	0	0	131
28.35 (Davis)	27.41–127 (Red Elongate) $(2n+1)$	86.1	185	20	1	1	0	0	17	224
29.42 (Davis)	27.41–127 (Red Elongate) $(2n+1)$	80.5	72	12	0	1	0	0	0	85
29.43 (Davis)	27.41–88 (Red Elongate) $(2n+1)$	64.9	81	11	1	0	1	0	0	94
30.31 (Davis)	29.42–75 (Red Elongate) $(2n+1)$	77	92	20	0	0	0	0	0	112
30.32 (Davis)	29.43–61 (Red Elongate) $(2n+1)$	57.2	98	10	0	0	1	0	0	109
31.31 (Anderson)	30.31–88 (Red Elongate) $(2n+1)$	45.8	98	4	0	0	0	1	29	132
31.32 (Anderson)	30.31–90 (Red Elongate) $(2n+1)$	27.3	41	0	0	0	0	0	40	81
31.33 (Anderson)	30.31–107 (Red Elongate) $(2n+1)$	74.7	114	2	0	0	0	1	20	137
31.34 (Anderson)	30.31–108 (Red Elongate) $(2n+1)$	84.5	121	8	0	1	0	1	16	147
31.35 (Anderson)	30.32–45 (Red Elongate) $(2n+1)$	86.3	252	15	1	3	0	0	42	313
32.01 (Anderson)	31.35–285 (Red Elongate) $(2n+1)$	60.8	23	3	0	0	0	0	0	26
32.02 (Anderson)	31.35–103 (Red Elongate) $(2n+1)$	87.8	178	1	0	0	0	0	1	180
32.03 (Anderson)	31.35–171 (Red Elongate) $(2n+1)$	68.7	46	1	0	0	0	0	0	47
32.05 (Anderson)	31.33–108 (Red Elongate) $(2n+1)$	81.2	227	3	0	0	0	0	3	233
32.06 (Anderson)	31.35–302 (Red Elongate) $(2n+1)$	89.03	181	18	1	0	0	2	3	205
32.07 (Anderson)	31.34–142 (Red Elongate) $(2n+1)$	73.26	45	3	0	0	0	0	0	48
Total			2319	144	4	13	12	5	205	2702

Cytological material was collected by Professor Davis in 1927, 1929, and 1930, and given to the writer for study. In addition to this material, the writer made large collections in 1931.

TABLE 3. *Reciprocal crosses between O. franciscana and Red Elongate*

Culture	Parents	% germination	O. franciscana	Red Elongate	Bushy Dwarf	Pointed Tips	Misc. Dwarf	Runts	New types	Died	Total
31.36 (Anderson)	(30.22–78 × 30.31–90) (O. franciscana × Red Elongate)	95.4	860	0	0	0	0	0	4	13	877
31.37 (Anderson)	(30.31–90 × 30.22–788) (Red Elongate × O. franciscana)	61.3	127	6	0	1	1	0	0	12	147
31.38 (Anderson)	(30.22–788 × 30.31–108) (O. franciscana × Red Elongate)	96.9	524	1	0	0	1	0	0	4	530
31.39 (Anderson)	(30.31–108 × 30.22–788) (Red Elongate × O. franciscana)	77.0	116	4	0	0	0	0	0	13	133
32.08 (Anderson)	(31.35–163 × 31.35–162) (Red Elongate × O. franciscana)	74.16	75	3	0	0	2	0	0	0	80
32.09 (Anderson)	(31.34–138 × 31.34–137) (Red Elongate × O. franciscana)	84.6	54	2	0	1	0	0	0	1	59
32.10 (Anderson)	(31.34–143 × 31.34–144) (Red Elongate × O. franciscana)	74.5	63	5	0	1	0	0	0	0	69
32.11 (Anderson)	(31.34–134 × 31.34–137) (Red Elongate × O. franciscana)	74.8	78	2	0	0	0	0	1	1	82
32.13 (Anderson)	(31.34–142 × 31.34–144) (Red Elongate × O. franciscana)	80.5	63	1	0	0	0	0	1	1	66
32.14 (Anderson)	(31.34–145 × 31.34–144) (Red Elongate × O. franciscana)	79.2	108	6	0	1	0	0	0	1	116
32.15 (Anderson)	(31.33–132 × 31.33–131) (O. franciscana × Red Elongate)	69.77	218	0	1	0	0	0	0	1	220
32.16 (Anderson)	(31.35–162 × 31.35–163) (O. franciscana × Red Elongate)	72.7	229	0	0	0	0	1	0	5	235

Bushy Dwarf appeared as 9.7 per cent of the total in the series of 13 cultures given in table 1, and Red Elongate as 5.3 per cent of the total of 19 cultures given in table 2. The death rate of seedlings in the cultures of Red Elongate (cultures 28.35–32.07, table 2) was 7.6 per cent; of Bushy Dwarf, 6.3 per cent (cultures 27.43–32.21, table 1). This seems to indicate greater viability of Bushy Dwarf seedlings, as germination rates were ap-

TABLE 4. *Reciprocal crosses between O. franciscana and Bushy Dwarf*

Culture	Parents	% germination	O. franciscana	Bushy Dwarf	Red Elongate	Pointed Tips	Misc. Dwarf	New types	Died	Total
30.36 (Davis)	(29.61–5 × 29.44–215) (O. franciscana × Bushy Dwarf)	96.9	286	0	0	0	0	0	0	286
30.37 (Davis)	(29.44–215 × 29.61–5) (Bushy Dwarf × O. franciscana)	59.2	119	15	0	1	0	0	0	135
31.43 (Anderson)	(30.22–788 × 30.35–110) (O. franciscana × Bushy Dwarf)	93.0	533	1	0	0	0	0	29	563
31.44 (Anderson)	(30.35–110 × 30.22–788) (Bushy Dwarf × O. franciscana)	80.8	200	4	0	0	0	1	22	227
31.45 (Anderson)	(30.22–788 × 30.35–112) (O. franciscana × Bushy Dwarf)	91.0	406	0	0	2	1	2	44	455
31.46 (Anderson)	(30.35–112 × 30.22–788) (Bushy Dwarf × O. franciscana)	90.5	248	36	0	2	1	1	34	322
32.22 (Anderson)	(31.42–145 × 31.42–144) (Bushy Dwarf × O. franciscana)	72.89	64	0	0	1	0	0	0	66
32.23 (Anderson)	(31.42–3 × 31.42–4) (Bushy Dwarf × O. franciscana)	56.9	19	3	0	0	0	0	1	23
32.24 (Anderson)	(31.46–315 × 31.46–228) (Bushy Dwarf × O. franciscana)	77.5	176	19	0	0	1	1	1	198
32.25 (Anderson)	(31.42–144 × 31.42–145) (O. franciscana × Bushy Dwarf)	77.7	245	0	0	0	0	0	0	245
32.26 (Anderson)	(31.41–60 × 31.41–61) (O. franciscana × Bushy Dwarf)	85.5	343	0	0	0	1	1	1	346
32.27 (Anderson)	(31.41–228 × 31.41–226) (O. franciscana × Bushy Dwarf)	84.9	197	0	0	0	0	0	0	197
32.28 (Anderson)	(31.41–238 × 31.41–237) (O. franciscana × Bushy Dwarf)	84.6	162	0	0	0	0	0	0	162
32.29 (Anderson)	(31.31–104 × 31.31–103) (O. franciscana × Bushy Dwarf)	94.6	321	0	0	1	0	0	0	322
32.30 (Anderson)	(31.35–294 × 31.35–297) (O. franciscana × Bushy Dwarf)	83.78	400	0	0	1	2	0	2	405
32.31 (Anderson)	(31.41–120 × 31.41–121) (O. franciscana × Bushy Dwarf)	95.6	212	0	0	0	0	0	1	213
32.32 (Anderson)	(31.44–204 × 31.44–226) (O. franciscana × Bushy Dwarf)	82.9	301	0	1	0	0	0	1	303
32.33 (Anderson)	(31.46–162 × 31.46–161) (O. franciscana × Bushy Dwarf)	87.08	762	4	0	0	2	0	3	771

proximately the same—68.7 per cent for Red Elongate and 71.5 per cent for Bushy Dwarf. This difference in the relative percentages of the appearance of the two sports is not entirely accounted for by differential seedling vigor. The number of dwarf rosettes and runts is twice as great in cultures of Red Elongate selfed as in cultures of Bushy Dwarf selfed, indicating an initial loss of Red Elongate forms through possible chromosome disarrangement.

In January, 1931, seeds subjected to freezing weather were germinated in Petri dishes following treatment of pressure and exhaust employed by Davis (1915). The cultures were from Red Elongate and Bushy Dwarf selfed and from both variants backcrossed to *franciscana*. Particularly large crosses were grown of the back crosses where Red Elongate and Bushy Dwarf had been used as the male parent (cultures 31.36, 31.38, 32.15, and 32.16, table 3; 31.43, 31.45, 32.27, and 32.33, table 4) to determine whether or not the high degree of male sterility was total. Germination following the treatment described above took place immediately, and the method has the advantage over earth-sown cultures in that it gives an accurate check on germination through the residue of ungerminated seeds that may be examined.

Cytological collections were made at all stages of development of anthers and ovaries. Anthers were fixed according to the following treatments, the dehydration being always through a long series of alcohols: (1) acetic acid-absolute alcohol (1:3), fixed not over 15 minutes; (2) Allen's modification of Bouin,[2] 6 hours, washed 24 hours; (3) strong Flemming, 24 hours, washed 24 hours; (4) Gilson's with 1 per cent acetic acid,[3] fixed 6 hours followed by 50 per cent, 60 per cent, 70 per cent, 85 per cent alcohols; (5) La Cour's (1929), 12 to 24 hours, washed 24 hours; (6) Nawaschin (Karpechenko),[4] 6 to 12 hours; (7) Telyesniczky, aqueous (Lee, 1928), 24 to 48 hours, washed 12 hours; (8) Zenker's, 6 hours, washed 12 hours; (9) Zirkle (1928), sulphuric acid-formaldehyde, various lengths of time up to 12 hours, thorough washing; (10) Zirkle,[5] formic acid-acetaldehyde-picric acid, fixation 48 hours, long washing. Material of ovaries was fixed in acetic-acid-absolute alcohol (1:3), Allen's modification of Bouin, strong Flemming, strong Flemming diluted one-half, Gilson's with 5 per cent acetic acid,[3] Nawaschin (Karpechenko),[4] Telyesniczky (aqueous), and La Cour's. Gilson's with 5 per cent acetic acid [3] was used most extensively, the fixation being for 6 hours, followed by treatment with 50 per cent, 60 per cent, 70 per cent, and 85 per cent alcohols. Whenever aqueous fixing fluids were used in treatment of anthers, it was found expedient to brush the anthers with water to hasten penetration.

[2] Allen's Modification of Bouin: Bouin's fluid, 100 cc.; chromic acid, 0.5 g.; urea, 2.0 g.

[3] Modified Gilson's with 1-5 per cent acetic acid: Bichloride of mercury (sat. sol. in 40 per cent alcohol), 100 cc.; nitric acid (c.p.), 2 cc.; glacial acetic acid, 1-5 cc.

[4] Nawaschin (Karpechenko): (A) Chromic acid, 4 g.; glacial acetic acid, 40 cc.; water, 240 cc. (B) Formalin, 160 cc.; water, 140 cc.

[5] Zirkle's formula (suggested through correspondence): Formic acid, 2 cc.; acetaldehyde, 6 cc.; picric acid (sat. aqueous), 92 cc.

In the case of ovaries an attempt was made to cut the material in pieces so small that penetration would be rapid.

Experience with mixtures so varied in nature impresses one with the fact that there is no fixing fluid uniformly superior for all phases of *Oenothera* meiosis. It was in general true that Nawaschin's [4] fluid gave rather better results in the fixation of anthers providing it was changed after the first hour. Gilson's with 1 per cent acetic acid, both from the view of relatively little shrinkage and brilliancy of staining following its use, rated next to Nawaschin's. Strong Flemming and Allen's modification of Bouin [2] were both satisfactory, but better differentiation was obtained in the contraction stages following treatment with the first two named. The acetic acid-alcohol (1:3) fixation proved rather disappointing, as staining after its use was unsatisfactory even if the sections were mordanted in Telyesniczky's fluid over night, as Weier (1930) suggests. Zirkle's sulphuric acid-formaldehyde fluid did not work at all well (Zirkle, 1928). His formic acid-acetaldehyde-picric acid was satisfactory but not better than the others, with the added difficulty involved in using the rapidly volatilizing acetaldehyde. Gilson's fluid with 5 per cent acetic acid proved most satisfactory in the case of studies of the ovary, although strong Flemming and Allen's modification of Bouin gave good results; but Flemming, diluted one-half, was very unsatisfactory. La Cour's (1929) fluid gave indifferent or erratic results; shrinkage following its use differed widely, depending on the phases studied in the sections treated. Shrinkage was evident in all material of both anthers and ovaries, regardless of the reagent, but varied greatly in degree.

Anthers were cut from 3 to 13 microns thick, the majority at 10 microns. Ovaries as a rule were cut at 11 microns but up to 20 microns when necessary.

Iron-alum-haematoxylin as usual was very satisfactory. For sections of ovaries thicker than 14 microns safranin counter-stained with light green according to the method of Blackman and Welsford (1913) proved useful. Crystal violet in 0.5 per cent aqueous solution according to the following schedule, Newton's modification of the Gram method, gave excellent results. After five to fifteen minutes in crystal violet the slides were rinsed in water, allowed a minute in 1 g. potassium iodide + 1 g. iodine in 100 cc. 80 per cent alcohol, and then passed very rapidly through 95 per cent and absolute alcohols. The preparations were cleared in a mixture of clove oil and xylol (1:3), where final differentiation took place, and followed by xylol to remove all of the clove oil.

No success attended attempts to germinate pollen in various concentrations of sugar. The addition of saliva or diastase to the media, as suggested by Tischler (1927), did not help. Sugar media with crushed stigmas were also tried, but without success.

THE CYTOLOGY OF RED ELONGATE

Somatic mitosis

Material excellent for studies on somatic mitosis was furnished by the anther walls and the nucellus of the developing ovule. The resting nucleus (fig. 1, pl. 22) has several nucleoli, only one of which is large. Within the nucleus is a fine network in which are distributed chromatic bodies. These bodies frequently number fifteen, which is the number of somatic chromosomes present. However, counts ranging from eleven to seventeen have been made; so it is not true that the number of these bodies consistently correspond to the number of chromosomes.

During prophase there is a rapid thickening of the threads (fig. 2, 3, pl. 22) which, however, does not proceed uniformly until the formation of the spireme has taken place (fig. 4). The crosswise segmentation of the spireme, partially indicated in figure 4, results in the formation of fifteen chromosomes, as shown in figure 5. This same figure shows lengthwise splitting of the chromosomes. These chromosomes are rod-shaped, of varying lengths, and somewhat bent at this stage. The chromosomes then move into the metaphase position, a polar view of which is given in figure 6. Still retaining the form of bent rods, the daughter chromosomes move to their respective poles (fig. 7), and the daughter nuclei are organized, with the chromosomes at first clearly evident (fig. 8). An early coarse network is then developed and is later replaced by a finer one, the nucleoli reappear, and the resting stage is again present (fig. 9).

Microsporogenesis

The pollen mother cells usually occur in two rows, but occasionally as a single row. The nucleus is slightly oval in outline and measures on an average 10.9 microns through the longer axis. Together with the large nucleolus are found deeply staining bodies numbering fifteen as a rule, but smaller counts are frequently met, and occasionally counts of sixteen and seventeen have been determined (fig. 10, 11, 12). Endonucleoli may be observed as in figure 16. A very fine network seems to be present from the earliest stages. This network does not stain very readily, but may be distinguished (fig. 10–13).

As the nucleus approaches prophase of meiosis, a thickening of the strands of the network takes place together with an elongation of the small oval bodies found in the earlier stages (fig. 13, 14). Later, spherical granules appear more or less uniformly distributed along the threads (fig. 15, 16, 17). The figures from 16 to 24 indicate a system of threads rather than a reticulum. This would suggest that the reticulate appearance in the earliest stages is due to the adsorption of stain at points where crossed threads have become joined in the process of fixation.

The thread system is at first made up of strands of uniform diameter

(fig. 16, 17), but later some threads appear to thicken at the expense of others which become attenuate. This finally results after synizesis in a relatively simple and much shorter thread system, as illustrated in figures 20, 21, and 22. While the thread system is still delicate, the nucleolus passes to the periphery of the nucleus, and the chromatic thread system contracts into the synizetic knot (fig. 19). Usually this knot is formed at the periphery of the nucleus on the side nearest the anther wall, but in many cases it is not. This would seem to indicate that other factors aside from the movement of the fixing fluids must be concerned in its position. The knot varies in density with different fixing agents. For example, fixation with acetic acid-absolute alcohol or careful fixation with Nawaschin's fluid leaves the knot least dense, while strong Flemming and Allen's modification of Bouin draw the threads into a dense mass. Telyesniczky's (aqueous) fluid left the nuclear structure so contracted that it was practically impossible to study. The same fluids act in about the same way on second contraction figures. As stated, the knot varies with the use of different reagents; but the fact that it does occur with all of the fixing fluids suggests that it is a natural process and not wholly an artifact, although it is intensified by poor fixation.

It is evident that the apparently reticular chromatic structure of the early phases gives way to a thread system. Sections cut at 13 microns show this rather clearly (fig. 20, 22). The thread system on emergence from synizesis is much shorter and consists of thicker threads that have lost their spherical chromatic bodies. Finally the thread system expands uniformly throughout the nucleus to give the stage of the hollow spireme (fig. 21). The history of prophase shows the consistent development of a thread system, and the earlier appearance of a reticulum (fig. 15) probably results from imperfect fixation by which crossed threads become united at points.

Looping of the spireme (fig. 20, 21, 22) is evident after synizesis, and figures 23-27 show that this looping becomes very conspicuous as second contraction approaches. Preparations sometimes show an apparently homogeneous mass of chromatin, but such conditions are certainly the result of imperfect fixation. Undoubtedly such a mass is made up of a thread system, but the cohesion of the threads is such that the system is hidden. That each of six loops represents a pair of chromosomes, while the seventh loop is made up of three chromosomes, is a logical interpretation. Such loops, as Weier (1930) has shown in *Oenothera Hookeri*, result from contraction of laterally paired threads and not from spireme segmentation. Gates and Goodwin (1931) apparently support Weier's conclusions in observations on meiosis in *Oenothera blandina*. Interlinked simple rings do not appear in either Red Elongate or Bushy Dwarf as they do in both *blandina* and *purpurata*. The chromosome pairs are free from the earliest observations possible after emergence from the second contraction.

Evidence for parasynapsis from chiasma formation is clearly indicated in figures of Catcheside (1931b). Crossing-over has been established by

Shull (1930) between the form *old gold* and *bullata.* Emerson (1931b) found crossing-over between homologous parts of dissimilar chromosomes in a circle of ten in *franciscana-sulfurea.* If crossing-over is correlated with chiasma formation and chiasma formation with parasynaptic behavior, there is reason to expect that further direct evidence for parasynapsis will be found.

Stages of diakinesis were numerous and easily studied. Figures 30 and 31 illustrate configurations of seven pairs and a univalent and six pairs and a trivalent, respectively. The pairs occurring at diakinesis are of two types. In one there is only a connection at one end (fig. 30 *a*), while in the other (fig. 30 *b*) there is one at each end, making of the two chromosomes what Darlington terms a " simple ring." When these pairs become arranged on the spindle at metaphase, the first condition gives rise to the dumbbell form *a* and the second to the shield form *b*, as shown in figure 33. Counts of a large number of diakinesis figures established the fact that the usual arrangement consisted of 4 simple rings, 2 horseshoe-shaped figures, and a trivalent or 4 simple rings, 3 horseshoe-shaped figures, and a univalent. At metaphase this same formation was carried out with the appearance of 4 shield-shaped pairs, 2 rods, and a trivalent or 4 shield-shaped pairs, 3 rods, and a univalent. That is, the shield-shaped pairs result when the spindle fibers are attached to the mid-section of the chromosome, and the rod- or dumbbell-shaped pairs when the point of attachment of the spindle fiber is near terminal.

Multipolar spindles (fig. 32) precede the bipolar structure at metaphase. The chromosomes move from diakinesis to metaphase without changing form, so that rings, pairs, univalents, or trivalents may all be in evidence at metaphase. The more common configuration is that illustrated by figure 33.

During anaphase the odd chromosome of a trisome accompanies one of its homologues. If the odd chromosome is present as a univalent, it frequently lags and usually lies with its longer axis at right angles to the spindle. Ordinarily there is only one lagging chromosome (fig. 34), although occasionally two were observed. Such chromosomes apparently do not undergo division, or if they do, there is no separation of the halves. No disintegration was noticed, rather a loss in staining capacity. No evidence was found indicating that chromosomes were ejected into the cytoplasm outside of the spindle.

The first evidence of lengthwise splitting of the chromosomes appeared during telophase (fig. 36, 37), no split being observed during anaphase (fig. 35). The split becomes very pronounced during interkinesis (fig. 38, 39, 40).

As indicated in the foregoing account, the distribution of chromosomes is 7 and 8 where no lagging takes place. No 6 and 9 distributions were found, although a great many nuclei were studied. During interkinesis the halves of the split chromosomes draw away from each other at the ends, giving to each pair the appearance of a Maltese cross (fig. 39, 40). Later the chro-

mosome halves become more attenuate (fig. 41). Counts made at inter-
kinesis bear out the statement regarding the frequency of lagging. Of 384
nuclei examined in the stage of interkinesis, 8 chromosomes appeared in only
135 nuclei instead of the expected 192. Care was taken to make this count in
such pairs of nuclei as occurred in the same pollen mother cell.

The homeotypic division of meiosis takes place quickly. Figures 42 and
43 show two metaphases from the same sporocyte with 7 and 8 chromosomes,
respectively. In figure 44 an early anaphase and a polar view of metaphase
are shown. Lagging in the second division anaphase also occurs. Figure
45 presents a type that is common; the halves of a split chromosome fail to
separate and degenerate in the central region of the spindle. This later
lagging during the homeotypic division naturally results in further lowering
the final number of 8-chromosome microspores formed. During telophase
a chromatic network is developed through the elongation and anastomosing
of the chromosomes (fig. 46). There was no indication of polyspory. Pol-
len grains were of two kinds, the good ones plump and well filled, the bad
ones shrunken and finally without cell contents. Among the shriveled grains
there were some not so much shrunken and possessing four instead of three
lobes (fig. 88). These are believed to result from cells containing 8 chromo-
somes. The percentage of bad pollen varied slightly with different plants,
but apparently not with the season, whether early or late. The largest propor-
tion of bad pollen recorded was 58 per cent and the lowest 43 per cent.

Megasporogenesis

The megaspore mother cell lies deeply imbedded in the nucellus and
becomes evident about the time when the integuments begin to form. The
resting nucleus (fig. 47) exhibits one or more nucleoli and a variable number
of chromatic bodies in a delicate reticulum. Some of these bodies are
probably chromosome centers, but the number is not at all times in accord
with the count of chromosomes in somatic tissues. A delicate thread system
then develops (fig. 48), the material of the chromatic bodies apparently pass-
ing into this reticulum. This stage is soon followed by the stage of synizesis
(fig. 49), which differs from the comparable figure in microsporogenesis in
that the knot is less dense in structure.

Figures 50, 51, and 52 illustrate emergence from synizesis, with the
formation of a thick spireme rather evenly distributed through the nucleus.
A contraction of the spireme then takes place (fig. 53–56), but is not so
marked in character as in the corresponding stage in the pollen mother cell.
Darlington (1931a) suggests that the extreme contraction in *Oenothera* is
due to the singleness of structure of the thread. However, the structure of
the thread in nuclei of both micro- and megasporocytes is the same, and yet
the degree of contraction is markedly different. The explanation of the
contraction must then involve other factors. Following the apparent seg-
mentation of the thread, the stages of diakinesis appear as illustrated in figures

57 and 58. It is interesting to note that the simple ring structures which appeared so often in microsporogenesis occur relatively infrequently. As a result the chromosomes at metaphase and anaphase (fig. 59–62) are found usually as rod-shaped pairs evidently developed from the horseshoe-shaped bivalents of diakinesis. A discussion of the matter will be presented later.

Counts of chromosomes made at diakinesis and at later stages established the number as fifteen. Figure 63 shows a 9–6 distribution of chromosomes which was not observed in the heterotypic division in microsporogenesis, but which is not infrequent here. Figures 64 and 65 illustrate a 7–7 and an 8–7 distribution, respectively. Lagging of chromosomes is usual, but only about half as frequent as in corresponding stages of microsporogenesis. The splitting of the chromosomes during interkinesis and the subsequent formation of X-shaped figures is less evident than in microsporogenesis. Interkinesis is of short duration and is often so rapidly followed by the homeotypic division that a wall between the dyads is not formed before the second division is well under way (fig. 66, 67, 68). Figure 68 shows a form of lagging frequently observed where a chromosome lies close to a daughter nucleus, having failed to become incorporated in it.

The final result of these two divisions is the formation of a tetrad of contiguous cells but of three distinct types. In some no walls appear between the megaspore nuclei (fig. 70); in others the walls are thin (fig. 71); and in a third type the walls are of considerable thickness (fig. 69, 73). The thick walls may possibly prevent fertilization of the embryo sac and subsequent seed development, resulting in some loss of fertility. These walls would not, however, act selectively against a particular type of megaspore, since all cells in the tetrad have the same heavy walls. Hence the wall formation has no significance in connection with the type of sterility found in these variants.

Only one megaspore nucleus survives, the other three breaking down. Ishikawa (1918) described the degeneration of the two center cells before their complete formation and the later degeneration of either the chalazal or micropylar cell. In this material no degeneration takes place until after all four cells are formed. In a count of 81 young embryo sacs 55 had developed from the cell at the chalazal end and 26 from that at the micropylar end. Occasionally the spores at both ends begin to form embryo sacs simultaneously, but only one continues development to maturity.

The surviving megaspore nucleus then moves to the micropylar end of the cell which is to become the embryo sac, and the lower portion of this cell becomes vacuolate (fig. 74). A group of 4 gametophyte nuclei is formed by two successive divisions giving rise to a pair of synergids (s), an egg nucleus (e), and a polar nucleus (p), as seen in figure 75. Usually the polar nucleus is the largest of the four. Large vacuoles appear between the synergids and the egg nucleus.

THE CYTOLOGY OF BUSHY DWARF COMPARED WITH RED ELONGATE

The studies made on Bushy Dwarf were as detailed as were those on Red Elongate. Morphologically the variants differed greatly, as shown in part by figures 1 and 2. Cytologically, however, meiosis in one runs parallel to that of the other, so much so that to present a complete series of figures for Bushy Dwarf would be a repetition of figures given for Red Elongate. Therefore only a few figures of stages representative of the similarity of the development will be presented.

The resting nucleus in the microsporocyte (fig. 76), like that in Red Elongate, contains several nucleoli and a delicate reticulum in which are imbedded chromatic bodies varying in number. Early prophase stages following the resting condition show the reticulum much more distinctly, one nucleolus and the gradual disappearance of the chromatic bodies. The disappearance is associated with the incorporation of the chromatin into the strands of the network (fig. 77). As synizesis approaches, the thread system becomes more marked, granular bodies appear on the threads, the nucleolus moves to the periphery, and a gradual contraction of the thread system takes place (fig. 78). On emergence from synizesis there is a thickening of the threads and a gradual filling of the nucleus with a system of threads now uniform in diameter, accompanied by a movement of the nucleolus toward the center of the nucleus (fig. 79). Later the threads thicken and become looped until at second contraction there is evident a configuration consisting of a relatively dense mass from which radiate loops, usually seven in number (fig. 80). Diakinesis figures which follow second contraction show as a rule a linear arrangement of the members of the trisome and six pairs of chromosomes. Occasionally the members of the trisome form a closed ring (fig. 81). In about half of the material seven pairs and a univalent were found. The multipolar spindle figure following diakinesis was again observed as in Red Elongate. Here again there is a preponderance of the shield-shaped chromosomes, indicating that at diakinesis the bivalent chromosomes occur more frequently as simple rings. Anaphase figures confirm the chromosome count of fifteen made in the earlier phases (fig. 83, 84). A lagging chromosome lying at right angles to the spindle fibers is illustrated in figure 84. This is the position which even at metaphase marks a chromosome as one which will lag. An attempt to relate the number of times that lagging took place to the occurrence of the odd chromosome as a univalent at diakinesis showed no correlation. The number of times the univalent lay at right angles to the spindle fibers at metaphase position was very closely correlated with the amount of lagging observed. Interkinesis figures in the two variants are similar (fig. 85), as are the homeotypic divisions which follow rapidly after interkinesis. This is illustrated by figure 86. The lagging of chromosomes during the homeotypic mitosis again reduces the numbers of 8-chromosome microspores. As in Red Elongate, polyspory was not observed.

Megasporogenesis in the two sports follows the same line of development

with two minor exceptions. Thick-walled tetrads were much less in evidence in Bushy Dwarf than in Red Elongate. This may account in part for the better seed production in Bushy Dwarf, since the presence of the thick walls may act as a deterrent to the fertilization of the embryo sac. The second peculiarity is the fact that in Bushy Dwarf the micropylar megaspore apparently functions with the same frequency as the chalazal megaspore in the development of the embryo sac, whereas in Red Elongate the latter seemed to be favored.

<div align="center">DISCUSSION</div>

Several years ago Blakeslee (1921) outlined some results from work done on *Datura Stramonium,* and expressed the hope that certain of the findings might be helpful in the solution of the more difficult problems involved in the studies on *Oenothera.* The mutant called Globe appeared as the first of a series of forms differing markedly from one another and from the parent *Datura Stramonium.* After cytological study had established the fact that the peculiarities of the Globe variant were associated with the presence of an odd chromosome, the assumption was made that eleven more sports should make their appearance, due in each case to the duplication of a different chromosome of the 12 in the haploid set. This assumption was sustained by the later appearance of these eleven additional variants readily distinguished by morphological peculiarities. All of the twelve mutants were found to produce gametes with 12 and 13 chromosomes; bad pollen grains appeared in relatively large proportions; rarely was the new character transmitted through the pollen and only to about one-fourth of the offspring through the egg. The explanation of 12 distinct variants necessitated the further assumption that it was not only the presence of an odd chromosome which brought about the mutation, but that a duplication of a specific chromosome of a set was essential for the expression of a particular mutation complex. Fortunately, although the number of Mendelian characters with which *Datura* is supplied are few, sufficient of these were found to make possible a check on the mode of inheritance and on the chromosome carrier.

After the discovery of the twelve expected trisomic variations additional · ones appeared, thus complicating what was apparently a simple situation. This resulted in the division of this type of variant into what Blakeslee terms primaries and secondaries. The primaries are distinguished from the secondaries, according to Blakeslee (1924), on the basis of both breeding behavior and the structure of the chromosomes. An example of breeding behavior is the fact that primaries occur spontaneously with much greater frequency than do secondaries. Furthermore, while secondaries occasionally come from their primaries, the latter arise regularly from their secondaries. The structure of the odd chromosome in the secondaries is believed to involve, as a rule, the duplication of a part. In one case (Blakeslee, 1924) the secondary resulted from the deficiency of a part. If the chromosomes of the trisome are AB, AB, AB, synapsis should show a pair and a univalent or a

chain of three. Should there be through segmental interchange a duplication and loss such that the chromosomes are AAB, B, AB, a ring might result as in secondaries. By a return of the segment A to the B chromosome the secondary would reproduce the primary AB, AB, AB. An instability of the AAB, B, AB condition may explain why secondaries throw back large proportions of primaries. Although this behavior would not involve the segmental interchange between non-homologous chromosomes (Belling and Blakeslee, 1924; Belling, 1925, 1927), the principle of the transfer of a portion of one chromosome to another is much the same. In *Datura* this duplication or deficiency of chromosome parts would account for the fact that secondaries appear to be modifications of their primaries. The peculiarities of the secondaries, according to Blakeslee, are not due to Mendelian factors.

In addition to secondaries, $2n + 1 + 1$ and $2n + 1 + 1 + 1$ forms have appeared in *Datura*—that is, two or more duplications of members of the haploid set have occurred. Theoretically this duplication might take place until all of the combinations between the diploid ($2n$) and triploid ($3n$) materialize. In *Datura* there have not been found forms with more than three additional chromosomes. McClintock (1929) in *Zea Mays* has reported duplication of as high as seven chromosomes of a haploid set of ten. These forms show an increase in the abnormal behavior with each additional odd chromosome, and this fact has an important bearing on the subject of meiotic irregularities met with in Red Elongate and Bushy Dwarf. The same increase in irregularity also has been observed in the odd chromosome variants of *Datura*.

As early as 1921 Blakeslee suggested that the variants of the Globe type had counterparts in the *lata* type of mutant found in *Oenothera*, with the exception that no chromosome characteristics of size or form have been found to show that the peculiarities of mutant *lata* are due to the presence of an odd chromosome of a particular set. Each of the two variants, Red Elongate and Bushy Dwarf, exhibits the odd chromosome, and the mutant character is transmitted as in *Datura* variants but in lesser degree. There is apparently complete male sterility in the *Oenothera* sports with respect to the $n + 1$ gamete, while transmission of the mutant character through the pollen of the Globe variant of *Datura* occurs in two per cent of the cases. Furthermore, there is a difference in the amount of bad pollen formed. While bad pollen in *Datura* variants is 7.9 per cent in the Globe and reaches only to 20.7 per cent in the variant Spinach, in Red Elongate and Bushy Dwarf the shriveled pollen ranges from 43 to 58 per cent.

The relation between nuclear disturbance and sterility has been established. A greater viability of n spores over $n + 1$ spores may be expected and less irregularity in the meiotic behavior of $2n + 1$ than in $2n + 1 + 1$ sports. Nuclear disturbance also may be comparatively greater in such variants as Red Elongate and Bushy Dwarf, in which one duplicate chromosome is added to the basic seven of the haploid set, than in *Datura* primaries, in which the addition of the odd chromosome is to a haploid set of twelve.

A corresponding difference exists between the number of $n + 1$ megaspores functioning in *Datura* sports and those in Red Elongate and Bushy Dwarf. In Globe the mutation complex in selfed forms is transmitted to about 25 per cent of the offspring and in Poinsettia to about 30 per cent. In the forms Red Elongate and Bushy Dwarf the transmission is to 15.1 and 19.4 per cent, respectively. Selective action against the $2n + 1$ zygote has been held responsible for some of the deficiency, but the difference in expected ratios may be associated with a possible greater disturbance brought about by the addition of the extra chromosome to the smaller seven-chromosome set of *Oenothera*. The difference in the greater viability of the $n + 1$ megaspore over the $n + 1$ microspore is perhaps attributable to the fact that its nutrition is better, and because it is better protected from the disturbance of environmental factors.

From the similarity of the behavior of the two variants to that exhibited by the *Datura* sports it seems quite possible that five more primaries will arise from *Oenothera franciscana* in addition to Red Elongate and Bushy Dwarf, since there are seven chromosomes in the haploid set. It may also be assumed that secondaries will occur such as have been found in *Datura*. The appearance of variants in which there is a duplication of more than one chromosome of the haploid set as in *Datura* is more problematical. The suppression of the $n + 1$ microspores seems to be complete; so any $2n + 1 + 1$ form would have to come from the fertilization of an $n + 1 + 1$ egg by a normal male gamete. Although a 9–6 chromosome distribution in megasporogenesis is not uncommon, no 16-chromosome variant has yet been isolated. The first appearance of Red Elongate and Bushy Dwarf was from the selfing of a haploid. Since $n + 1$ male gametes are apparently not formed, it may be assumed that the variant in each case resulted from the fertilization of an $n + 1$ female gamete by an n-chromosome male gamete. The $n + 1$ egg resulted from non-disjunction in the homeotypic division of the haploid following the suppression of the heterotypic mitosis. The incidence of mutation is about $1 : 1200$ for Red Elongate and $1 : 2000$ for Bushy Dwarf, as indicated by the occurrence of these sports when the cultures arise from *franciscana* pollinated by the variants. As far as the two variants are concerned, the writer is inclined to hold with the opinion expressed by Bartlett (1915) that in *Oenothera* no $n + 1$ male gamete functions. An apparent exception to this principle was encountered in culture 31.38, table 3, and culture 31.43, table 4, in each of which a single mutant appeared, the only cases out of some 3000 recorded for the back-cross, *franciscana* pollinated by the $2n + 1$ variants. It is possible that these two mutants were derived from $n + 1$ eggs of *franciscana*, the result of non-disjunction.

Buchholz and Blakeslee (1930) attribute the gametic sterility in primary mutations of *Datura* to selective action at germination of the pollen, selective action against the $n + 1$ gamete as exhibited by the differential rate of pollen tube growth, and bursting of pollen tubes, presumably those carrying the

$n + 1$ gametes. This would place the agencies responsible for sterility in the group of environmental factors. Goodspeed (1929) had already suggested that the production of fertile gametes in plants depends on nicely balanced physiological factors arranged in a particular manner in the living organism, and that numerous agents, either environmental or inherent, may upset this balance. Watkins (1932) has prepared a table of style and pollen tube chromosome relationships in various material which would indicate that the elimination of $n + 1$ gametes takes place due to faulty adjustments. That is, so long as the chromosomal relation between the stylar tissue and that of the pollen tube remains $2n$ and $1n$, there are no difficulties. Just as soon as the relation becomes as $2n$ and $1n + 1$, maladjustments result in selective action against the $n + 1$ pollen tube. It does not seem to the writer necessary to go beyond the actual formation of pollen grains in these two variants of *Oenothera* to account for the sterility, which involves, not the functioning of the $n + 1$ male gamete, but its non-formation.

The facts regarding extensive lagging of chromosomes have been given. Counts do not show the elimination of every 8-chromosome microspore prior to the tetrad stage, and the appearance of 4-lobed grains (fig. 88) somewhat larger than the normal grains seems to be significant. These pollen grains are found collapsed among the bad pollen grains when free pollen is examined (fig. 89). Harrison and Blackburn (1927) in their studies on pollen of roses found just such conditions obtaining in pollen with additional chromosomes. Michaelis (1926) in his work on *Epilobium angustifolium* found a relationship existing between chromosome number and morphological structure. The 4-lobed grains may then be those with the eight chromosomes.

Attempts to germinate *Oenothera* pollen by artificial means were not successful. Observations of stigmas which had been uniformly pollinated showed the germination of all good grains, indicating that if there were any 8-chromosome pollen grains among them they were not to be recognized through failure to germinate.

Referring back to the statement of Buchholz and Blakeslee (1930) that selective action at germination of pollen is one of the causes of sterility, it might be expected that delayed growth of pollen tubes of Red Elongate and Bushy Dwarf would be more pronounced than in *Datura* variants, since the odd chromosome is added to the smaller set of seven in the *Oenothera* sports instead of to the twelve in *Datura*. However, the apparent absence of the $n + 1$ male gamete in the two variants dealt with in these studies appears more likely to be explained by the non-formation of a large number of possible $n + 1$ microspores because of the lagging of the chromosomes and to the abortion of any 8-chromosome microspores, probably represented by the 4-lobed pollen grains.

The limited number of 8-chromosome megaspores in *Oenothera* is quite marked, the percentages for Red Elongate and Bushy Dwarf being 15.1 and 19.4, respectively. These figures were obtained by taking the ratio of the

41

total number of such variants appearing through selfed plants to the total number expected. In contrast with these percentages, Gates (1923) reported fertility as high as 50 per cent in his $2n + 1$ variants. Some of this low fertility may be accounted for by selective elimination of the $2n + 1$ zygote, and perhaps some is due to similar action against the female $n + 1$ gamete. Most of it is attributable to the loss of the odd chromosome in the meiotic processes. Lagging, although frequently met with in megasporogenesis, is not as extensive as it was in microsporogenesis.

The spindle fiber attachment to the chromosomes at metaphase of megasporogenesis was usually not at the mid-region, as had been the case in microsporogenesis. Odd chromosomes with the mid-region attachment were the ones which failed to be incorporated in the daughter nuclei at heterotypic division. Whether or not this may be taken as an indication of the loss of homology in the odd chromosome such that it fails to pair with its former homologues presents an interesting question. It is assumed in *Datura* that the odd chromosome may be modified either by duplication of a part or a deficiency of a part such that mutants known as secondaries result. A modification might be such as is represented by a change in the point of attachment of the spindle fibers. This theory of a change that will modify the degree of attraction between homologous chromosomes offers an hypothesis for the difference in the output of $n + 1$ microspores and $n + 1$ megaspores in *Oenothera*. The very dense second contraction figure of microsporogenesis offers a much greater opportunity for structural modification than does the looser figure found in the corresponding stage of megasporogenesis. On this basis, then, the odd chromosome in microsporogenesis which has its spindle fiber connection at the mid-region may be interpreted as one which has changed to such an extent that it fails to move to the pole, but remains at its metaphase position. The type of univalent having a terminal connection with its spindle fibers is apparently capable of becoming one of the members of the set of the daughter nuclei, at least during the dyad stage. It was not possible in the studies made to trace such a chromosome through the homeotypic division where lagging occurs, but it may be assumed that whatever lagging does take place involves one of the six halves of the members of the trisome which appeared at heterotypic metaphase.

Parasynapsis as the mode of meiosis has been assumed. Weier (1930), from studies of meiosis in *Oenothera Hookeri* with seven pairs of chromosomes, reports zygotene threads which, becoming shorter and thicker during pachynema, were counted as seven in number. During second contraction they appear as seven arms radiating from the central coagulum and are to be considered as bivalents resulting from parasynapsis. He concludes that *Oenothera Hookeri* presents meiotic behavior parasynaptic in character. In *Oenothera Lamarckiana* Weier finds pairing only between two threads representing, as he suggests, the single pair of chromosomes which appears at diakinesis in this species. The other twelve chromosomes are represented by

twelve free threads at this stage which become later a chain of twelve chromosomes. Gates and Goodwin (1931) present corroborative evidence of lateral pairing of threads from studies of meiosis in *Oenothera blandina* and *Oenothera purpurata,* two species exhibiting seven pairs of chromosomes, as in *Oenothera Hookeri.* These investigators conclude that the spireme, hitherto interpreted as being continuous, is not in that condition at any time in forms with paired chromosomes. The apparent continuity of the spireme is interpreted as due to the fact that interlinking of chromosome pairs takes place. The loops appearing at second contraction represent such interlocking, each loop consisting of a chromosome pair. If the figures descriptive of heterotypic mitosis in Red Elongate and Bushy Dwarf are compared with those presented by Weier (1930) and Gates and Goodwin (1931), the similarity is marked. Weier's " free arms " and the loops described by Gates and Goodwin are the same structurally. Evidence of conjugation is difficult to obtain, but in addition to that presented above, Emerson (1931b) shows presynaptic conjugation of pachynemal threads in an unknown species of *Oenothera,* a species having pairs instead of chains of chromosomes.

Darlington (1931), enlarging on his investigations of an earlier date (1929), presents another type of evidence for parasynaptic behavior in *Oenothera.* Assuming that studies on phases earlier than diakinesis meet with unusual technical difficulties, he reinvestigated the later stages. The appearance of double connections between the homologous ends of chromosomes reported by him indicates the terminalization of chiasmata between halves of the chromosomes. These chiasmata were formed in stages prior to diakinesis, indicating the presence of chromatids in those stages, a condition characteristic of parasynapsis. The two connections have not been frequently observed, possibly because they may fuse when imperfectly fixed and appear as one strand. A study of trivalents supports Darlington's conclusions, since, when trivalents occur as Y-shaped groups, the triangle formed by the connections does not collapse readily. Håkansson (1930) has found such conditions in the trisomic forms *curta* (a secondary from *cana*) and mutant *lata,* both *lata* and *cana* being mutants of *Oenothera Lamarckiana.* Emerson (1931a) presents, in his figures 287–6, 288–81, and 287–5 of an undetermined *Oenothera,* terminal connections which give evidence of earlier chiasma formation. In Red Elongate and Bushy Dwarf the presence of the trivalent offers no assistance, since it occurs in a chain form and not as a Y-shaped group, so that figures such as Håkansson (1930) presents in his studies are not possible.

Catcheside (1931b) gives the most convincing evidence of chiasma formation in studies on a form having a ring of 6 chromosomes and 4 pairs and believed to be a half-mutant from *Oenothera Lamarckiana.* The form shows lateral pairing, both in the chromosomes of the ring and in the free pairs. The difference in the pairing seems to be one of degree—that is, the chromosomes of the free pairs lie together along the entire length, while those

in the rings associate only at homologous segments. Demonstrations of chiasma formation were obtained beginning with late diplotene and in diakinesis and the heterotypic metaphase.

The above evidence for chiasma formation, together with the demonstration of crossing-over in *Oenothera* by Shull (1930) and Emerson (1931b), presents a strong argument for a parasynaptic interpretation of meiosis in *Oenothera*. If chiasmata are formed as the mechanism for crossing-over and are characteristic only of parasynaptic behavior, it is probable that the method of meiosis in all Oenotheras will be found to be parasynaptic.

SUMMARY

1. The appearance of two primary trisomic variants, Red Elongate and Bushy Dwarf, is reported from an *Oenothera* with pairing chromosomes. The sports originated from the selfing of a haploid (Pointed Tips), which in turn had its origin as a sport from *Oenothera franciscana*.

2. Bushy Dwarf appeared in the ratio of 1:422 from the haploid selfed (cultures 24.25 and 26.26, table 1), and Red Elongate appeared in the ratio of 1:41 from similar selfing (cultures 24.25, 26.25, and 27.41, table 2). In crosses in which the variants furnished the pollen with *franciscana* as the female parent (tables 3 and 4), the incidence of mutation was 1:1260 for Red Elongate and 1:2000 for Bushy Dwarf. Red Elongate appeared only twice in all of the cultures from Bushy Dwarf selfed, a total of 1634 plants. Bushy Dwarf, on the other hand, arose five times among 2173 plants, the progeny of Red Elongate selfed. Tables 1 and 2 record the occurrence of the haploid, Pointed Tips, six times in cultures of selfed Red Elongate (1:200) and twice in the cultures of Bushy Dwarf selfed (1:600).

3. When Bushy Dwarf and Red Elongate were pollinated by *franciscana*, the former appeared as 8.4 per cent of the total and the latter as 3.4 per cent (tables 3 and 4). Only one plant of each sport (culture 31.38, table 3; culture 31.43, table 3, 4) occurred in cultures in which *franciscana* had been pollinated by Red Elongate and Bushy Dwarf, respectively. These probably resulted, not from the fertilization of an *n-franciscana* egg by an $n+1$ male gamete, but from the fertilization of an $n+1$ egg by a normal male gamete. The $n+1$ female gamete probably arose by non-disjunction during megasporogenesis in the *franciscana* parent.

4. Each variant exhibits from 43 to 58 per cent shriveled pollen. There are no giant pollen grains in the pollen, but 4-lobed ones somewhat larger than the normal grains are found. These grains later shrivel, and it is suggested that they carry the 8 chromosomes.

5. Each variant gave rise to forms differing from the parents. There were dwarfs which did not mature and three new types which matured but which have not yet been studied.

6. A cytological study of the variants revealed similar behavior on the part of the two sports. In microsporogenesis a study of the earliest phases

showed a resting nucleus with a delicate thread system, in which were found a variable number of chromatic bodies. The contention of Leliveld (1931) that they represent pairing bodies was not substantiated.

7. The looping of threads between the hollow spireme stage and second contraction is very conspicuous. The loops present at second contraction are seven in number. Six are interpreted as consisting of pairs of chromosomes and the seventh as a trisome.

8. Diakinesis, metaphase, and anaphase figures give the count of fifteen as the chromosome number. The odd chromosome appears either as a univalent or as a member of a trisome, the trisome being a chain. The univalent has its spindle fiber connection either at mid-section or near-terminal. As a rule those of the former type are the ones which lag at heterotypic division. Lagging is common at both divisions, so that as a result very few 8-chromosome microspores are formed. The distribution at the heterotypic division of microsporogenesis is 8 and 7, no 9 and 6 distribution having been observed.

9. In megasporogenesis the meiotic process parallels that in microsporogenesis, except that lagging is less frequent and a 9 and 6 distribution of chromosomes occasionally occurs. Four types of megaspore tetrads are formed, ranging from those with thin walls to those with thick walls. In Red Elongate the chalazal megaspore seemed to be favored over the micropylar one, but in Bushy Dwarf each has about the same chance of development.

10. The $n + 1$ megaspores are not formed as numerously as expected. Their deficiency is largely due to lagging of chromosomes, thus preventing the formation of this type of megaspore. There may in addition be some selective action against the $n + 1$ gamete and the $2n + 1$ zygote. The presence of the $n + 1$ female gamete is probably explained by the much less pronounced lagging of chromosomes in megasporogenesis associated with a smaller degree of environmental maladjustment.

11. The variants Red Elongate and Bushy Dwarf correspond to primaries in *Datura*. There are certain peculiarities of behavior, as, for example, the greater degree of sterility, in which the *Oenothera* and *Datura* sports differ; but this condition may be the result of greater nuclear disturbance occasioned by the addition of an odd chromosome to a haploid set of seven in *Oenothera*, as contrasted with the addition of an odd chromosome to a basic set of twelve in *Datura*.

12. The meiotic behavior in the two variants is interpreted as parasynaptic.

The investigation was carried on under the direction of Professor B. M. Davis, who suggested the problem, furnished certain material from his garden, and gave permission to incorporate from his records such data as were necessary for a complete history of the forms studied. The friendly

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DESCRIPTION OF PLATES

All figures, unless otherwise noted, were sketched with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm. (num. aper. 1.30) in combination with the compensating ocular 20 ✕, giving a magnification in the field of the microscope of 2400 diameters. Figures have been reduced to one-half in reproduction.

PLATE 22

Figures 1–9. Somatic mitosis in Red Elongate. Fig. 1. The resting nucleus with chromatic bodies. Fig. 2, 3. Early prophase showing rapid thickening of the thread. Fig. 4. Segmenting spireme. Fig. 5. Differentiation of fifteen chromosomes some of

which show lengthwise fission. Fig. 6. Equatorial plate, view from the pole of the spindle. Fig. 7. Early telophase. Fig. 8. Organization of daughter nuclei with chromosomes still in evidence. Fig. 9. Daughter nuclei with chromatic bodies.

Figures 10–27. Microsporogenesis in Red Elongate. Fig. 10–12. Resting nuclei of microsporocytes with chromatic bodies and a delicate thread system. Fig. 13, 14. Early prophase stages showing material passing from the chromatic bodies into a thread system. Fig. 15–17. Prophase stages illustrating a fairly uniform thread system bearing granules. Fig. 18. Presynizesis figure illustrating the thickening of some threads at the expense of others, the threads, however, still thin. Fig. 19. Mid-synizesis. Fig. 20–22. Thickening of the threads and shortening of the thread system after emergence from synizesis. The hollow spireme is exhibited in figure 21. Fig. 23–26. Conspicuous looping during the stages immediately before second contraction. Fig. 27. Second contraction.

PLATE 23

Figures 28–45. Microsporogenesis in Red Elongate. Fig. 28, 29. Emergence from second contraction. Fig. 30, 31. Diakinesis, 30 a, an open ring from which will come the chromosome arrangements a of figure 33. 30 b, a closed ring which at metaphase gives rise to a shield-shaped pair as b of figure 33. Fig. 32. Multipolar spindle. Fig. 33. Metaphase showing at the right a trisome. a, a dumbbell-shaped pair derived from an open ring as a of figure 30. b, a shield-shaped pair derived from a closed ring as in b of figure 30. Fig. 34. Anaphase with one lagging chromosome. Fig. 35. Polar view of late anaphase, the chromosomes of which show no split. Fig. 36, 37. Telophases. A 7–8 distribution of chromosomes. Some indications of lengthwise splitting. Fig. 38–40. Interkinesis with split chromosomes. Fig. 41. Later interkinesis, the chromosomes forming a simple thread system. Fig. 42, 43. Homeotypic metaphases with 8 and 7 split chromosomes, respectively. Fig. 44. Homeotypic mitosis. Early anaphase in one figure and a polar view of metaphase in the other. Fig. 45. Lagging at homeotypic anaphase.

PLATE 24

Fig. 46. Microsporogenesis in Red Elongate. Tetrad. Development of chromatin network through elongation and anastomosing of the chromosomes. ✕ 160.

Figures 47–63. Megasporogenesis in Red Elongate. Fig. 47. Resting nucleus, chromatic bodies. Fig. 48. Early prophase with delicate thread system. Fig. 49. Mid-synizesis. Fig. 50, 51. Thicker thread system emerging from synizesis. Fig. 52. Thick spireme following synizesis. Fig. 53, 54. Approaching second contraction. Endonucleoli shown in figure 53. Fig. 55, 56. Second contraction illustrating loops, usually seven in number. Fig. 57, 58. Diakinesis with closed and open rings. Fig. 59. Heterotypic metaphase with the univalent and seven pairs of chromosomes. Fig. 60. Heterotypic metaphase with trivalent and six pairs of chromosomes. Fig. 61. Early anaphase with fifteen chromosomes. Fig. 62. Late anaphase showing 7–8 distribution of chromosomes. Fig. 63. Early telophase showing a 9–6 distribution of chromosomes, no splitting in evidence.

PLATE 25

Figures 64–73. Megasporogenesis in Red Elongate. Fig. 64. Late telophase illustrating a 7–7 distribution, chromosomes split. Fig. 65. Interkinesis illustrating 8–7 distribution. Fig. 66. Homeotypic metaphase. Fig. 67. Homeotypic anaphase with 14 and 16 chromosomes. Fig. 68. Homeotypic telophase illustrating lagging of one chromosome. Fig. 69–73. Types of tetrads formed as a result of homeotypic division, showing varying thicknesses of the cell walls. ✕ 160.

PLATE 26

Figures 74–75. Megasporogenesis in Red Elongate. Fig. 74. Megaspore. $\times$ 1200. Fig. 75. Female gametophyte. *s*, synergids, with vacuoles below. *e*, egg. *p*, polar nucleus. $\times$ 320.

Figures 76–89. Microsporogenesis in Bushy Dwarf. Fig. 76. Resting nucleus in microsporocyte, chromatic bodies. Fig. 77. Early prophase, formation of delicate thread system. Fig. 78. Mid-synizesis. Fig. 79. Hollow spireme following synizesis. Fig. 80. Second contraction showing seven loops. Fig. 81. Diakinesis with six rings and a trisome. Fig. 82. Heterotypic metaphase. Fig. 83. Early anaphase. Fig. 84. Late anaphase with a lagging chromosome. Fig. 85. Interkinesis, 7 and 8 split chromosomes. Fig. 86. Homeotypic metaphase divisions, an 8–7 distribution of chromosomes. Fig. 87. Normal 3-lobed pollen and two 3-lobed shriveled grains. $\times$ 80. Fig. 88. 4-lobed pollen grains believed to carry eight chromosomes. $\times$ 80. Fig. 89. Older 4-lobed grains now shriveled. $\times$ 80.
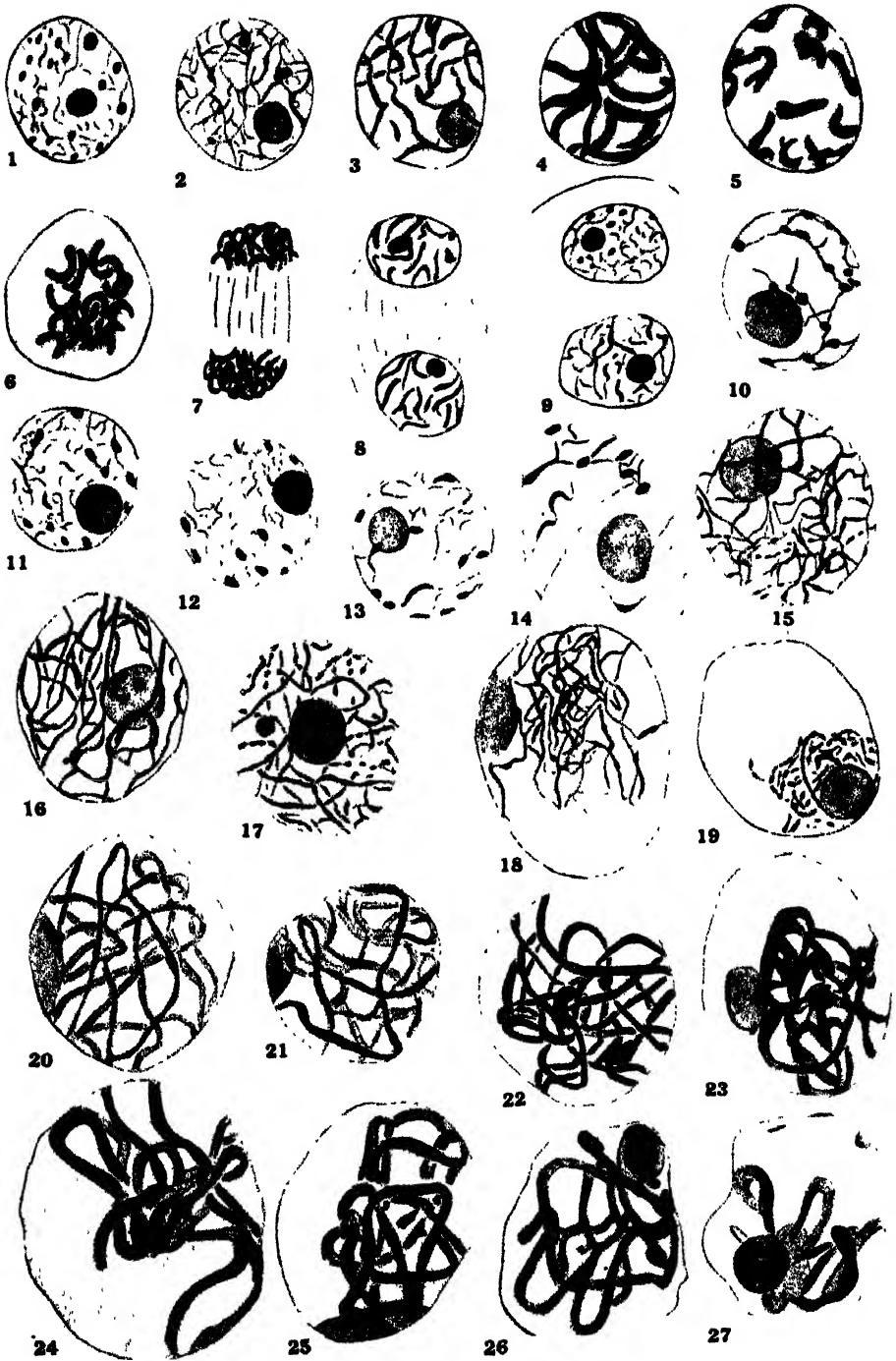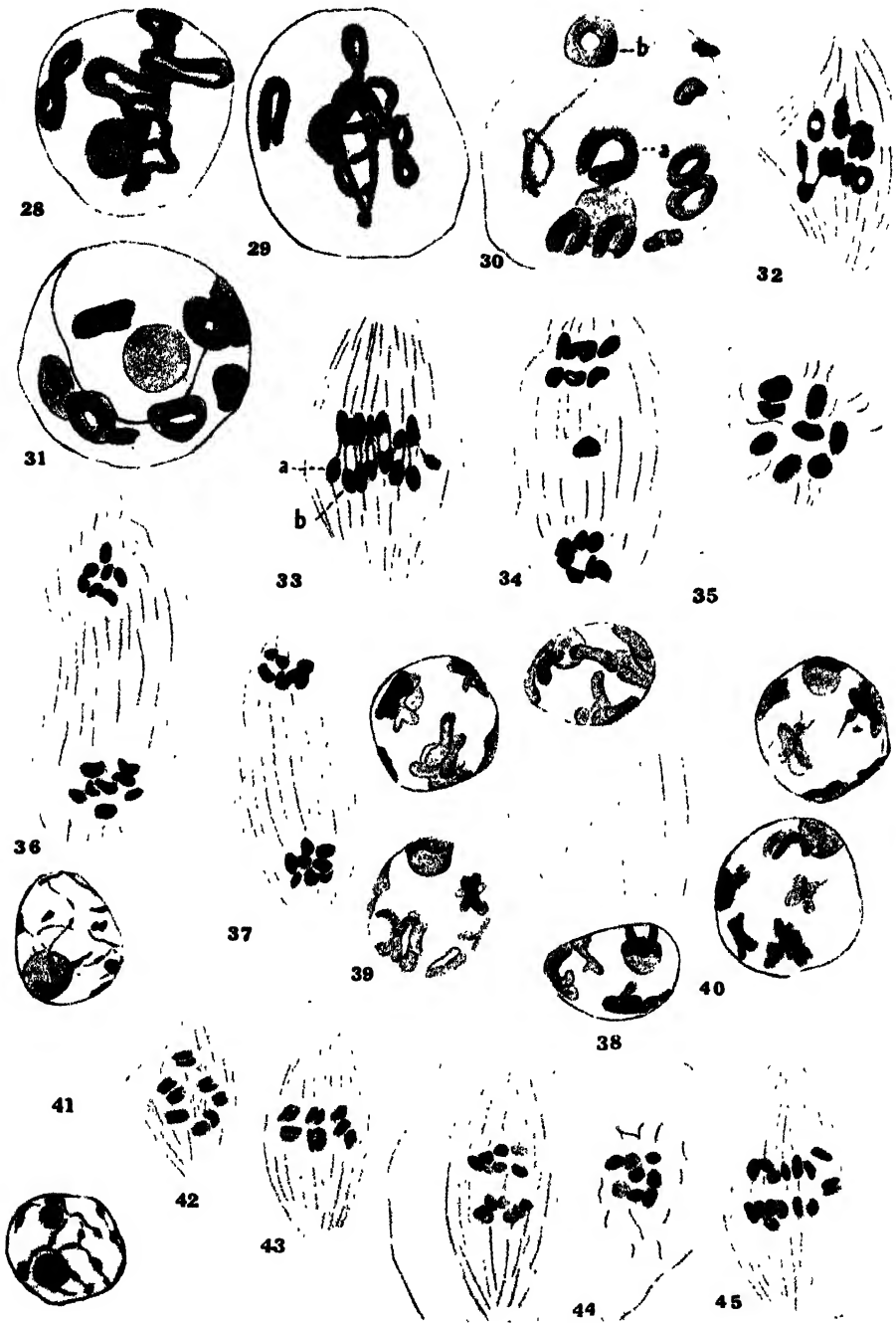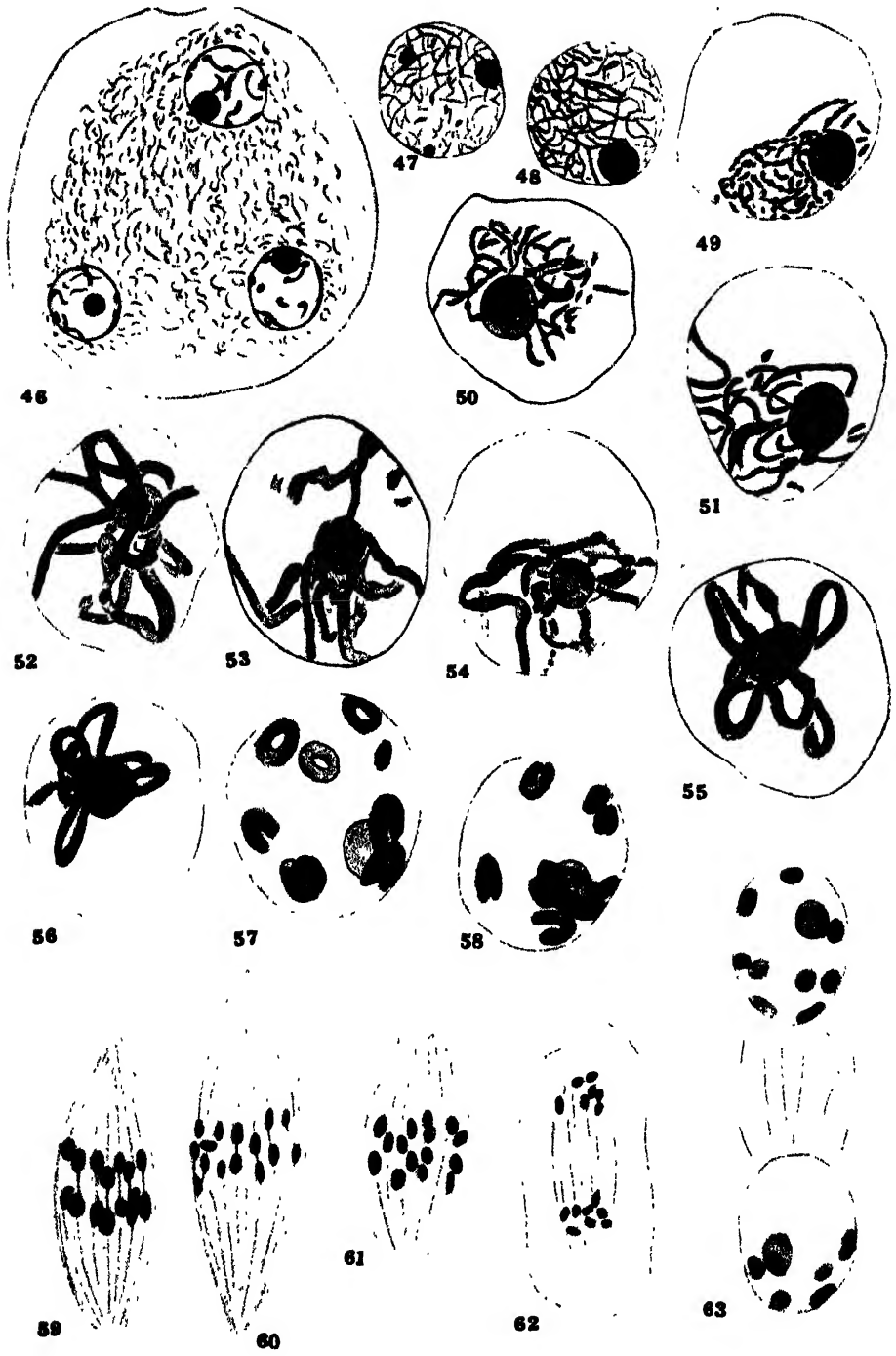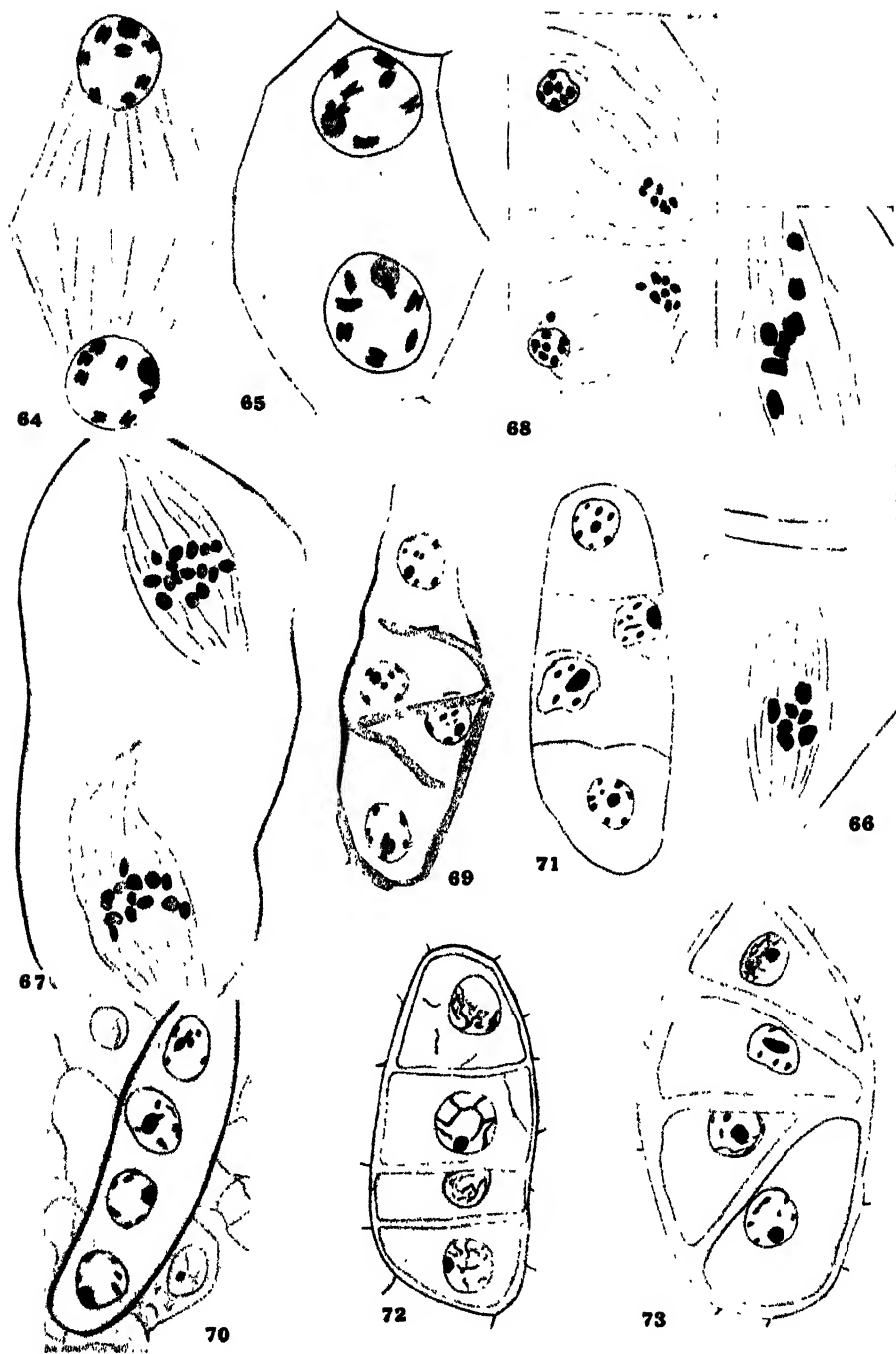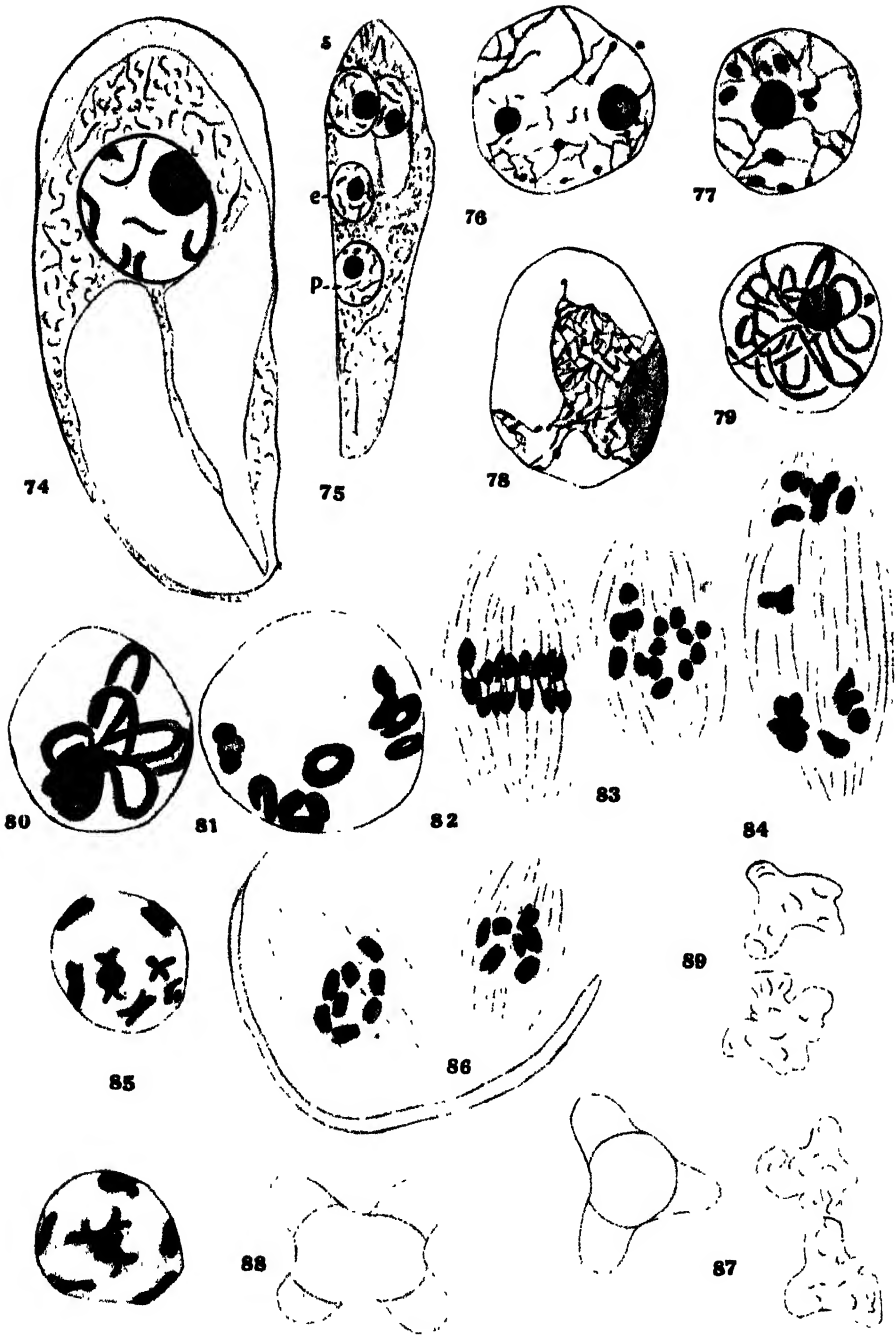
ANDERSON: OENOTHERA

A TAXONOMIC STUDY OF THE GENUS *NAMA*. I

C. Leo Hitchcock

HISTORY OF THE GENUS

Nama was proposed as a genus by Linnaeus in the Species Plantarum ed. 1, 226. 1753, based upon the species *N. zeylanicum*. Six years later (Syst. Nat. ed. 10, 2: 950. 1759) *N. jamaicense* was described; but in 1763 (Spec. Pl. ed. 2, 328. 1763) *N. zeylanicum,* the generic type, was transferred to *Hydrolea,* a procedure which caused several subsequent generic shifts and made it necessary for the International Botanical Congress of Vienna (Reg. Intern. Nomen. Bot. 89. 1906) to take action, by conserving the genus *Nama* to include *N. jamaicense.* This species, therefore, became the type of the genus.

Revisions of the genus have been published by Choisy (Mém. Soc. Phys. Genève 6: 111. 1833 and DC. Prodr. 10: 182. 1846), Gray (Proc. Am. Acad. 5: 337. 1861 and 8: 282. 1870; Hemsl. Biol. Cent.-Am. Bot. 2: 360. 1882), and Brand (Pflanzenreich 4^{251}: 142. 1913). The latter revision, in which 36 species and numerous subspecies, varieties, and forms were recognized, is the most thorough as well as the most recent treatment of the group.

The generic name *Conanthus* was published by Watson (Bot. King Exp. 256. 1871) in 1871, with the result that many of the species of *Nama* were transferred by Heller (Cat. N. Am. Pl. 6. 1898) to the newer genus. In 1891, Kuntze (Rev. Gen. Pl. 2: 434. 1891) proposed the name *Marilaunidium* for most of the species of *Nama* that were then known. He pointed out that *Nama,* as recognized by Linnaeus in 1753, was based upon a plant that had later been transferred to *Hydrolea.* His solution of the problem was to restrict the appellation *Nama* to the genus to which *N. zeylanicum* belonged. He, therefore, replaced the generic name *Hydrolea* by that of *Nama,* and renamed the aggregate of species that had been called *Nama* (including *N. jamaicense*), giving them the new name *Marilaunidium.* When the International Botanical Congress voted to preserve *Nama* as the genus which should include *N. jamaicense,* rather than *N. zeylanicum,* the followers of the American Code continued to use the name *Marilaunidium* in the light of Kuntze's interpretation.

In the treatment of the *Hydrophyllaceae* in the Pflanzenreich Brand erected a new genus *Andropus,* due to a misinterpretation of the nature of the filament bases of the plant in question. The latest attempt at generic clarifica-

tion was made by Macbride (Contr. Gray Herb. **49** : 42.   1917), who pointed
out the dissimilarity of *N. Parryi* to the other species of *Nama* and proposed
a new genus *Turricula* to include this plant.

It was because of the uncertainty of the generic status of *Turricula,
Andropus,* and *Conanthus,* and of the position of *N. Lobbii* and *N. Roth-
rockii,* as well as because of the difficulty one encounters in trying to interpret
Brand's treatment of the group, that the present study was undertaken.

ACKNOWLEDGMENTS

GENERIC RELATIONSHIPS

Nama has been given varied generic treatments.   Because of the united
styles of *N. aretioides,* Gray consistently maintained that species as a mono-
typic genus.   Brand, however (Pflanzenreich 4^{251}: 143.   1913), reduced
Conanthus to a subgenus with seven species.   It seems to the writer that
Conanthus can neither be maintained as a genus nor as a large subgenus.
The two species *N. aretioides* and *N. densum* are alike in having united
styles, pitted seeds, and unappendaged filaments.   They are, in addition,
habitally alike; they can rationally be preserved as a subgenus or section.
However, other species with united styles show little relationship to them.
Nama biflorum is very closely related to *N. jamaicense* and *N. spathulatum*
is equally closely related to *N. Palmeri,* whereas *N. stenocarpum* is distinct
enough, because of the semi-inferior ovary, to merit subgeneric or sectional
rank by itself.

Nama Lobbii and *N. Parryi* have been considered by such workers as
Greene (Bull. Calif. Acad. **1** : 202.   1885 and Pittonia **2** : 22.   1889) and
Hall (Univ. Cal. Pub. Bot. **1** : 106.   1902 and Zoë **5** : 265.   1908) to belong
to the genus *Eriodictyon.*   Of these two species, it seems to the writer that
N. Parryi should be included under *Eriodictyon* because of the habit, corru-
gated seeds, corolla (bearded within), and loculicidally and septicidally de-
hiscent capsules.   *Nama Lobbii,* however, may seemingly be most expedi-
ently kept in *Nama;* the habit is that of *Nama,* as are the corolla and seed
characters.   The capsule, which is somewhat cartilaginous and loculicidally

and septicidally dehiscent, is an *Eriodictyon*-character, however. It seems wise, therefore, to preserve *N. Lobbii* as a third section or subgenus of *Nama;* it is through *N. Lobbii* and *E. Parryi* that the true relationship of the two genera is manifest.

The capitate inflorescence and crenate leaves of *N. Rothrockii* are sufficient to warrant placing this plant in a separate section. The remaining species are homogeneous enough to be maintained in a fifth section, much the largest of the five.

Although *Nama,* as thus treated, includes a group of species diverse in character, it may quite easily be distinguished from the closely related genera of the *Hydrophyllaceae*. *Lemmonia* and *Draperia* both have but four seeds, and the species of *Eriodictyon* have loculicidally and septicidally dehiscent, cartilaginous capsules, and transversely corrugated seeds, and are much more woody than are any of the species of *Nama*.

TAXONOMY

NAMA Linnaeus Syst. Nat. ed. 10, 2: 950. 1759, not Sp. Pl. ed. 1, 226. 1753; Choisy, Mém. Soc. Phys. Genève 6: 111. 1833 and in DC. Prodr. 10: 182. 1846; Gray, Proc. Am. Acad. 5: 337. 1861 and in Hemsl. Biol. Cent.-Am. Bot. 2: 360. 1882; Benth. & Hook. Gen. Pl. 2²: 826. 1876; Peter in E. & P. Pflanzenf. 4³ᵃ: 68. 1897; Brand, Pflanzenr. 4²⁵¹: 142. 1913; Jepson, Man. Fl. Pl. Calif. 831. 1925. *Conanthus* Watson, Bot. King Exp. 256. 1871 (footnote); Peter, l. c. 65; Benth. & Hook., l. c. *Marilaunidium* O. Kuntze, Rev. Gen. Pl. 2: 434. 1891. *Andropus* Brand, Fedde Rep. Spec. Nov. 10: 281. 1912 and in Pflanzenr. 4²⁵¹: 162. 1913.

Herbaceous to suffrutescent, erect to prostrate, pubescent annuals or perennials. Leaves mostly alternate and entire. Flowers borne singly in axils or in reduced lateral or terminal cymes. Calyx divided nearly to base, the lobes linear-lanceolate to spatulate. Corolla tubular to narrowly obconic-campanulate, pubescent without. Stamens mostly included, subequal to unequal, with one exception unequally inserted; filament-bases various, usually somewhat dilated, the adnate portion with or without free margins, glabrous with one exception. Styles 2, usually free, but sometimes partially and incompletely united or nearly completely united. Ovary many-ovuled, 1-celled, but apparently 2-celled by the ingrowth of the placentae, pubescent with one exception. Capsule loculicidally, rarely also septicidally dehiscent, membranous (cartilaginous). Seeds numerous, variously pitted, alveolate or reticulate, to smooth, sometimes very minutely transversely corrugated as well as pitted.

Type species: *N. jamaicense* Linn. Syst. Nat. ed. 10, 2: 950. 1759.

Unusual species: *N. Lobbii* with pubescence on filament-bases, and cartilaginous capsules; *N. origanifolium* with stamens nearly equal and equally inserted, the bases without free margins; *N. Rothrockii* with crenulate leaves and capitate inflorescence; *N. serpylloides* with opposite leaves; *N. stenocarpum* with half-inferior ovary; *N. torynophyllum* with glabrous styles and ovary.

KEY TO SECTIONS

Calyx divided about ¾ of its length, the tubular portion adnate to ovary, the ovary thus
 partially inferior. ....Sect. IV. *Zonolacus.*
Calyx divided to base or nearly so, not grown to ovary.
 Styles permanently connate nearly to apex; small, matted annuals with unappen-
  daged or very minutely appendaged filaments. ....Sect. III. *Conanthus.*
 Styles free when dried, or if connate, plants not as above and filament-bases with
  free-margined adnate portion.
  Leaves crenate-dentate. ....Sect. I. *Cinerascentia.*
  Leaves not crenate-dentate, entire or nearly so.
   Capsule somewhat cartilaginous, loculicidally and septicidally dehiscent.
               Sect. II. *Arachnoidea.*
   Capsule membranous, loculicidally dehiscent only. ......Sect. V. *Eunama.*

SECT. I. CINERASCENTIA

Section Cinerascentia Peter, E. & P. Pflanzenf. 4^{3a}: 69. 1897.
Subgenus *Rothrockia* Jepson, Man. Fl. Pl. Calif. 832. 1925.

Leaves crenate-dentate; flowers in capitate inflorescences.

1. N. Rothrockii Gray, Bot. Calif. 1: 621. 1876, Syn. Fl. N. Am. 2^1:
175. 419. 1886; Brand, Pflanzenr. 4^{251}: 160. 1913. *Marilaunidium Roth-
rockii* (Gray) Kuntze, Rev. Gen. Pl. 2: 434. 1891. *Conanthus Rothrockii*
(Gray) Heller, Cat. N. Am. Pl. 6. 1898. Pl. 27, fig. 11.

A low spreading pilose-hirsute and hispid, glandular perennial, the branches
1–3.5 dm. tall, glandular and hispid with hairs as much as 3 mm. long; leaves
lanceolate or elliptic to oblong or oblong-oblanceolate, 2–6 cm. long, 0.3–1.5
cm. broad, coarsely and deeply crenate-dentate, somewhat revolute, short-
petiolate, strigose to pilose-hirsute and viscid or glandular; flowers numerous
in dense terminal heads; calyx-lobes linear, 10–13 mm. long, hirsute-hispid;
corolla narrowly obconic-campanulate, 13–16 mm. long; stamens unequally
inserted, free portion of filaments about equal to adnate portion, thickened
and dilated slightly from point of insertion to base of corolla, the margins
free, glabrous; styles 8–10 mm. long; capsules 16–20-seeded; seeds brown,
ca. 1.5 mm. long, finely but prominently alveolate.

Representative material—United States: California: Mt. Whitney, *Kellogg* (C) ;
Inyo Co.—Upper Bishop Creek, Andrew's Camp *K. Brandegee* (C, M) ; Monachi,
Rothrock 301 (G, Type), *Austin 164* (C). Tulare Co.—Big Arroyo on Kern R., *Cul-
bertson 4682* (C, G, M, P) ; Olanche Mt., *Hall & Babcock 5262* (C, G, M, P, S) ; Trout
Meadows, upper Kern R., *Hall & Babcock 5411* (C, G, S). Fresno Co.—Wood's
Creek, *Clemens in 1910* (P). Kern Co.—Kern R. Flat, *Culbertson in 1904* (P). San
Bernardino Co.—Holcomb Valley, *Parish & Parish 338 in 1882* (G, M, S), *Parish 338
in 1885* (C, M), *Munz 10634* (C, P).

SECT. II. ARACHNOIDEA

Section Arachnoidea Peter, l. c. Subgenus *Lobbiana* Jepson, l. c.

Leaves entire; capsules somewhat cartilaginous, loculicidally and septi-
cidally dehiscent.

2. N. Lobbii Gray, Proc. Am. Acad. 6: 37. 1862, Brand, l. c. 162. *Erio-
dictyon Lobbii* (Gray) Greene, Bull. Cal. Acad. Sci. 1: 202. 1885. *Conan-*

thus Lobbii (Gray) Heller l. c. *Marilaunidium Lobbii* (Gray) Kuntze, l. c.
Pl. 27, fig. 4.

A suffruticose, tomentose, very leafy perennial, 0.5–3 dm. tall; leaves
practically sessile, linear or narrowly elliptic to spatulate or even obovate,
those of older branches very numerous, 1–3 cm. long, 1.5–4 mm. broad,
glandular, glabrate above, sparingly lanate or floccose beneath, revolute, those
of juvenile branches and of flowering branches less numerous, some much
larger, 2.5–6 cm. long, 3–12 mm. broad, with fascicles of smaller leaves in
the axils, scarcely if at all glandular, arachnoid above, but densely lanate and
therefore grayish below, but little revolute; flowers crowded in densely leafy
reduced cymes, subsessile; calyx-lobes linear, 3–5 (7) mm. long, lanate;
corolla narrowly obconic-campanulate, ca. 10 mm. long; stamens unequal,
free portion of filaments, 3–4 mm. long, unequally inserted 2–3 mm. from
base of corolla, the adnate portion thickened and expanded, extending to base
of corolla, its margins free, each with 6–8 long hairs; styles ca. 3 mm. long;
capsules somewhat cartilaginous, loculicidally and septicidally dehiscent, 8–14-
seeded; seeds dark brown, nearly 1.5 mm. long, very minutely papillate-rugose.

Representative material—UNITED STATES: California: without locality, *Lobb 108*
(G, TYPE); near Sisson, Siskiyou Co., *Heller 8050* (G, M), *Setchell & Dobie* in 1902
(C, P); n. fk. Coffee Cr., Trinity Co., *Alexander & Kellogg 242* (C); lava beds of
n.e. Shasta Co., *Hall & Babcock 4234* (C, G, M); Lassen's Peak, Lassen Co., *Bruce
435* (M); near Lassen Buttes, Plumas Co., *Brown 655* (M); Sardine Lake, Sierra Co.,
Hall & Babcock 4490 (C, M, P); Jonesville, Butte Co., *Copeland 448* (C, M); Soda
Spgs., Nevada Co., *Jones 2496* (G, P); Truckee R., Placer Co., *Sonne 243* (M); Emer-
ald Bay, Lake Tahoe, Eldorado Co., *Noonan 27* (M), *Geis 41* (C). Nevada: Washoe
Co., without locality, *Hall & Chandler 4556* (C), Incline Road, *Kennedy 1472* (C, M).

SECT. III.   CONANTHUS

SECTION CONANTHUS (Wats.) comb. nov. *Conanthus,* as genus, Wats.
Bot. King Exp. 256. 1871 (footnote), as subgenus Brand, l. c. 143 and
Jepson, Man. Fl. Pl. Calif. 832. 1925.

Styles connate ¾ their length; small prostrate, dichotomously branched
annuals of United States.

Corollas 3–5 mm. long.
    Plant hirsute; leaves 8–15 mm. long, 1–3 mm. broad; corollas 3–4 mm. long.
                                                            3. *N. densum.*
    Plant hirsute-hispid; leaves 10–40 mm. long, 1.5–4 mm. broad; corollas 4–5 mm. long.
                                                            3a. *N. densum* var. *parviflorum.*
Corollas 7–16 mm. long.
    Moderately leafy; corollas 7–15 mm. long. ....4. *N. arctioides.*
    Densely leafy; corollas 12–16 mm. long. ....4a. *N. arctioides* var. *multiflorum.*

3. N. DENSUM Lemmon, Bull. Torr. Bot. Club 16: 222. 1889; Brand,
l. c. 144. *Conanthus densus* (Lemmon) Heller, l. c. *N. demissa* Wats. Bot.
King Exp. 259. 1871, in small part (*Lyall* specimen). *Conanthus parvi-
florus* Greenman, Erythea 7: 117. 1899, in part, as to specimens cited. Pl.
27, fig. 14.

A prostrate, hirsute annual, few-branched from base, the branches densely
leafy at apex, 2–8 cm. long; leaves narrowly spatulate, 8–15 mm. long, 1–3

mm. broad, obtuse or rounded, gray-hirsute; flowers solitary, sessile; calyx-lobes narrowly linear-lanceolate, ca. 2.5–3.5 mm. long; corolla tubular, 3–4 mm. long; stamens unequally inserted 1–1.5 mm. from corolla-base, the filaments ca. 0.3 mm. long, filiform, with minute divergent scales ca. 0.3 mm. long at base (the free margins of adnate portion); styles 0.3–1 mm. long, united at least ¾ their length; ovary 14–16-seeded; seeds dark brown, ca. 0.6 mm. long, shallowly pitted and minutely transversely corrugated.

Representative material—UNITED STATES: Nevada: without locality, *Gray in 1872* (G); Reno, Washoe Co., June 8, 1897, *Jones* (P); Washoe Lake, June 3, 1897, *Jones* (P); Candelaria, Mineral Co., *Shockley 382* (G). Oregon: 9 mi. n. of Crescent, Klamath Co., *Nelson 5022* (G). California: near Edgewood, Siskiyou Co., *Lemmon & Lemmon in 1889* (C, TYPE collection); Eagle Lake, Lassen Co., *Baker* (P); near Bronco, Nev. Co., May 20, 1888, *Sonne* (C); Mono Co., Leavitt's Meadows, Aug. 22, 1898, *Congdon* (G); Mammoth, *K. Brandegee in 1913* (C, P); Inyo Co., McAfee Meadow, White Mts., *Duran 566* (C, M, P); foothills w. of Bishop, *Heller 8325* (CA, G, M, S); Cottonwood Cr., *Purpus 1925* (C). The following collections are intermediate between the species and the variety *parviflorum:* Mill City, Pershing Co., Nevada, May 28, 1903, *Jones* (P); Belleville, Mineral Co., Nevada, *Shockley 340* (G).

3a. **N. densum** Lemmon var. **parviflorum** (Greenman) comb. nov. *Conanthus parviflorus* Greenman, Erythea **7**: 117. 1899, in large part. *Gilia hispida* Piper, Erythea **6**: 30. 1898, acc. to description and annotations.

Dichotomously branched, hirsute-hispid, the branches rather uniformly leafy, 3–15 cm. long; leaves elliptic-lanceolate to narrowly spatulate, 1–4 cm. long, 1.5–4 mm. broad, acute, greenish; corolla 4–5 mm. long; stamens unequally inserted 1.5–2 mm. from base of corolla, filaments ca. 0.5 mm. long; styles 1–1.5 mm. long.

Representative material—UNITED STATES: Idaho: Glenn's Ferry, Elmore Co., June 17, 1911, *Jones* (P, but not S). Utah: Thompson's Spgs., Grand Co., May 24, 1892, *Eastwood* (C, S); Westwater, Grand Co., May 6, 1891, *Jones* (P); Chepeta Well, May 23, 1908, *Jones* (P). Nevada: near Wadsworth, *K. Brandegee* (C). Washington: Wallula, Wash. Terr., *T. S. Brandegee 978* (G); Wash. Terr. *Canby 978* (C); plain bet. Pineville and Bear Buttes, *Leiberg 325* (CA, G, M, P, S); Jct. Crab and Wilson Creeks, Douglas Co., *Sandberg & Leiberg 259* (C, CA, G, M); Pasco, Franklin Co., *Piper 2968* (G). Oregon: sage plains, *Howell 492* (G); Malheur region, e. Oregon, *Cusick 1957*, TYPE collection (C, G, M, P in part only, US); Cottonwood Canyon, e. Oregon, *Leiberg 2072* (G); Pilot Butte, Crook Co., *Nelson 828* (G, M); Kimberly, Grant Co., *Henderson 5091* (CA, G, M, S). California: plains n. of Alturas, Modoc Co., *Austin 244* (C), *84* (P).

4. **N. ARETIOIDES** (H. & A.) Brand, Univ. Cal. Pub. Bot. **4**: 224. 1912, Pflanzenr. **4**²⁶¹: 143, fig. 27. 1913. *Eutoca aretioides* H. & A. Bot. Beech. Voy. 374. 1838; DC. Prodr. **9**: 295. 1845. *E. aretioides β pumila* H. & A., l. c. *N. aretioides* f. *californica* Brand, Univ. Cal. Pub. Bot. **4**: 224. 1912. *N. aretioides* var. *californica* (Brand) Jepson, Man. Fl. Pl. Cal. 832. 1925. *N. aretioides* f. *genuina* Brand, Pflanzenr. **4**²⁶¹: 144. 1913. *Phacelia (Coreanthus) aretioides* (H. & A.) Gray et al, Pac. R. R. Rep. **5**⁴: 22. 1860. *N. psammophilum* Goodman, Ann. Mo. Bot. Gard. **19**: 177. 1932 (merely an ecological variant). *Conanthus aretioides* (H. & A.) Wats. Bot. King Exp. 256. 1871. *Marilaunidium aretioides* (H. & A.) Coville, Contr. U. S. Nat. Herb. **4**: 161. 1893.

With the habit and general aspect of *N. densum*, the leaves similar, 1–2.5 cm. long, 1–5 mm. broad; calyx-lobes linear-lanceolate, 3.5–7 mm. long; corolla tubular-campanulate, 7–15 mm. long; stamens inserted unequally near middle of tube or below, filaments 1–2 mm. long, adnate portion without free margins; heterostyllous, the styles 2–6 mm. long, united to near apex; capsules 10–35-seeded; seeds similar to those of *N. densum*, the transverse corrugations somewhat deeper.

Representative material—UNITED STATES: Idaho: King Hill, Elmore Co., *Nelson & Macbride 1118* (C, G, M, S); Payette, Canyon Co., *Macbride 866* (G); Bruneau, Owyhee Co., *Jones 25570* (P); rim of Snake R. Canyon, near Hagerman, May 22, 1930, *Shoop* (M, TYPE of *N. psammophilum*); Glenn's Ferry, June 17, 1911, *Jones,* good match for preceding plant (P, S). Nevada: Hawthorne, Mineral Co., June 22, 1882, *Jones* (P); Carson City, June 2, 1897, *Jones* (P). Washington: near Morgan's Ferry, Yakima R., *Suksdorf 399* (G). Oregon: without locality, *Cusick* in 1885 (G, same as *Shoop* plant); Squaw Cr., near Humphrey Ranch, Grant Co., *Henderson 5360* (CA, G, S); Ontario, Malheur Co., *Leiberg 2016* (C, G); region of Snake R., e. Oregon, *Tolmie* (G, part of TYPE). California: without locality, *Torrey* in 1865 (G) and *Bolander* in 1872 (G); Sherwin Grade, Mono Co., *Feudge 1390* (P); w. of Independence, Inyo Co., *Austin 92* (C); Mt. Spring Canyon, Argus Mts., Inyo Co., *Purpus 5029* (C, G, M).

Nama arctioides is quite variable in leaf and flower size, but material from Washington, Idaho, and southwestern California is about the same, having smaller corollas and wider leaves than material from central California and western Nevada.

4a. N. ARETIOIDES var. MULTIFLORUM (Heller) Jepson, Man. Fl. Pl. Cal. 832. 1925. *Conanthus multiflorus* Heller, Muhlenbergia 2: 238. 1906. *N. arctioides* f. *multiflora* and f. *nevadensis* Brand, Univ. Cal. Pub. Bot. 4: 224. 1912.

Densely leafy; corollas 12–16 mm. long, the limb much expanded.

Representative material—UNITED STATES: Nevada: Reno, Washoe Co., May 27, 1903, *Jones* (P); Ormsby Co., Eagle Valley, *Baker 1025,* TYPE collection *N. a.* f. *nevadensis* (C, CA, G, M, P); near Empire City, *Torrey 341* (G), *Jones 3989* (CA, M, P); Candelaria, Mineral Co., *Shockley 226* (C, G, S in part); Esmeralda Co., May, 1881, *Shockley* (G); Pah-Ute Mts., Nye Co? *Watson 883* (G). California: Thousand Creek Flat, Humboldt Co., June 29, 1909, *Kellogg* (C); Honey Lake, Lassen Co., *Eisen* in 1891 (C); Sierra Co., *Lemmon 174* (M in part); Fort Bidwell, Modoc Co., May, 1878, *Austin* (G); w. of Bishop, Inyo Co., *Heller 8286,* TYPE collection *C. multiflorus* (C, G, M, S, US).

SECT. IV.   ZONOLACUS

SECTION ZONOLACUS (Jepson) comb. nov.; as subgenus Jepson Man. Fl. Pl. Calif. 832. 1925.

Styles connate, ovary partially inferior.

5. N. STENOCARPUM Gray, Proc. Am. Acad. 10: 331. 1875, Hemsl. Biol. Cent.-Am. Bot. 2: 365. 1882; Brand, Pflanzenr. 4[251]: 145. 1913. *Conanthus stenocarpus* (Gray) Heller, Cat. N. Am. Pl. 6. 1898. *Marilaunidium stenocarpum* (Gray) Kuntze, l. c. *N. undulatum* as treated by Gray, Proc. Am. Acad. 8: 282. 1870, in part. *N. dichotoma* var. *pauciflora*

Choisy ex Brand, l. c. (publ. in synonymy). *N. micrantha* Nees, ex Brand, l. c. (publ. in synonymy). *N. humifusum* Brand, Beiträge z. Kennt. d. Hydroph. (Königl. Gymnas. Sorau, Beilage z. Jahresb. 9. 1911). Pl. 27, fig. 6 and 16.

A rather leafy, ascending or erect (prostrate) strigose-hirsute annual, the branches 10–30 cm. long; leaves oblong to spatulate, 10–40 mm. long, 2–10 mm. broad, somewhat undulate, gradually attenuate to a short petiole, sessile or even clasping; flowers 2–3 at the nodes, and in short leafy terminal cymes, on pedicels 1–3 mm. long; calyx hirsute, tubular, the tube 2–4 mm. long, grown to ovary and not separable from the latter, the lobes linear to spatulate, 4–7 mm. long; corolla tubular-obconic, ca. 5 (7) mm. long; stamens subequally inserted about 1 mm. from corolla-base, adnate portion of filaments with rather conspicuous free margins (scales) that run to base of corolla, the scales widened upward and prolonged slightly beyond place of attachment to base of filament at its point of insertion on corolla; styles (very frequently 3) ca. 2 mm. long, united about ⅔ their length, but rather readily separable; capsules 120–250-seeded; seeds ca. 0.3 mm. long, straw-colored, their sides rather irregularly flattened, finely but conspicuously alveolate.

Representative material—UNITES STATES: Uvalde, Uvalde Co., Texas, *Palmer 12317* (C, M); Arizona, near Yuma, *Vasey* in 1881 (G); Colo. R., Nevada, *Lemmon 165* (G). California: Fort Yuma, *Thomas* (G); Colorado R., Yuma, *Lemmon 83* (G); Blue Lake, Colo. Desert, *Abrams 3196* (G, M, P, S); Soldier's Home grounds, Los Angeles Co., Oct. 1889, *Hasse*, TYPE, *N. humifusum* (C); Santa Monica, Oct. 1889, *Hasse*, probably part of above collection (G, US); near Santa Monica, Sept. 1889, *Hasse* (M, S); reservoir, Soldier's Home, L. A. Co., *Abrams 2572* (G, M, P, S); Orange Co. —Laguna Canyon, *Munz, Street & Williams 2681* (P); Laguna, *Abrams 1770* (S); Sweetwater Valley, San Diego Co., *Cleveland 352* (C, M). MEXICO: Gardner's Laguna, Baja California, *Schoenefeldt 2902* (S, US). Tamaulipas—without locality, *Berlandier* (M); Matamoras, *Berlandier 2328 = 898* (G, M), *2525 = 1095*, TYPE collection (G, M), *2126=709* (G), *2111 =694* (G, M); San Miguel between Laredo and Bejar, *Berlandier 1435=175* (G, but not M). Coahuila—near Parras, *Palmer 853* (G, M, US); Monclova, *Palmer 857* (G, US); Yaqui R., *Palmer 111* (G). Sinaloa—vic. of Guadalupe, *Rose, Standley & Russell 14689* (M, US). Durango—valley of Nazas, *Gregg 635* (M).

SECT. V. EUNAMA

SECTION EUNAMA C. L. Hitchcock sect. nov. Subgen. *Marilaunidium* (Kuntze) Brand, Pflanzenr. 4²⁵¹: 146. 1913. Section *Palaeonama* Brand, l. c. 147. Section *Neonama* Brand, l. c. 157. Subgenus *Neonama* Jepson, l. c. 832. Sections *Decurrentia, Amplexicaulia, Hispida, Spathisepala, Origanifolia*, and *Hirsuta* of Peter, E. & P. Pflanzenf. 4³ᵃ: 69. 1897.

Capsule membranous, loculicidally dehiscent; leaves entire; ovary superior; styles not united over ½ their length.

Perennials.
    Corolla 15–25 mm. long. ....9. *N. sericeum.*
    Corolla less than 15 mm. long.
        Ovary 4–12-seeded; leaves 1.5–8 cm. long, with long petioles. ...10. *N. hirsutum.*
        Ovary with more than 12 seeds (except no. 15?); leaves sometimes as much as
            4 cm. long, but if so, sessile or short-petiolate.
        Free portion of filaments shorter than the adnate portion.

Plant sericeous-villous; leaves 2–4 cm. long, 0.4–1.5 cm. broad; plants of Texas. ....17. *N. Havardii.*

Plant hirsute-hispid; leaves 1.5–2.5 cm. long, 0.2–0.4 cm. broad; plants of Mexico. ....11. *N. Purpusii.*

Free portion of filaments at least as long as the adnate portion.

Leaves mostly opposite. ....15. *N. serpylloides.*

Leaves prevailingly alternate.

Filaments equally inserted, the bases widened, without adnate portion.

Leaves ca. 7 mm. long, 1.5–4 mm. broad; corolla 4–6 mm. long. 12. *N. origanifolium.*

Leaves 13–25 mm. long, 4–15 mm. broad; corolla 6 mm. long. 12a. *N. origanifolium* var. *rupicolum.*

Filaments subequally or unequally inserted, with free-margined adnate portion.

Flowers borne on long filiform pedicels 4–12 mm. long or longer. Styles more or less connate, petioles wing-margined. 16. *N. biflorum.*

Styles free, petioles not wing-margined. 14. *N. rotundifolium.*

Flowers sessile or on short, rather stout pedicels 1–4 mm. long.

Seeds yellow; small, tufted plants, branches not over 10 cm. long; leaves scarcely over 1 cm. long; corolla 6–8 mm. long. ....13. *N. xylopodum.*

Seeds brown; more erect plants, branches 5–40 cm. long; leaves 1–4 cm. long; corolla 7–12 mm. long.

Filaments with adnate, widened, free-margined base nearly as long as free portion; leaves 1–3 mm. broad.

Plant yellow-green, strigose-hispid or -hirsute.

Leaves strigose-hirsute, mealy-granular. 8a. *N. stenophyllum* var. *flavescens.*

Leaves strigose-hispid, not mealy granular. 8b. *N. stenophyllum* var. *egenum.*

Plant grayish-green, hirsute-hispid. 8. *N. stenophyllum.*

Filaments with widened, free-margined bases much shorter than free portion; leaves 2–10 mm. broad.

Leaves more or less clasping, 4–10 mm. broad; corolla 7 mm. long; styles more or less connate. ....7. *N. spathulatum.*

Leaves not clasping, 3–7 mm. broad; corolla 9–12 mm. long; styles free.

Leaves silvery-sericeous, 2–4 mm. broad, strongly revolute; calyx-lobes not hardened in fruit. 6a. *N. Palmeri* var. *argenteum.*

Leaves not silvery-sericeous, 3–7 mm. broad, not strongly revolute; calyx-lobes hardened in fruit. ....6. *N. Palmeri.*

6. N. PALMERI Gray ex Hemsl. Biol. Cent.-Am. Bot. 2: 361. 1882; Brand, l. c. 148. *Marilaunidium Palmeri* (Gray) Kuntze, l. c. Pl. 27, fig. 20.

A suffrutescent, ascending, strigose- or silky-villous-hirsute or -hispid perennial, the branches rather woody, somewhat creeping, very leafy, 10–20 cm. tall; leaves linear-elliptic to oblong, linear-spatulate, or oblong-obovate, 1.5–3 cm. long, 3–7 mm. broad, very short-petiolate to sessile, more or less strigose-silky, glandular beneath, somewhat revolute; flowers in few-flowered dense clusters, subsessile or with petioles as much as 5 mm. long; calyx-lobes linear-lanceolate, 5–7 mm. long, enlarging and hardening in fruit; corolla narrowly obconic-campanulate, 9–12 mm. long; stamens inserted 1–2 mm. from corolla-base, free filaments somewhat flattened, enlarged toward base, widest just below point of insertion; styles 3–4 mm. long; capsules 12–40-seeded; seeds ca. 1 mm. long, ovoid, brown, reticulate-alveolate, the markings much like those of *N. biflorum.*

Material seen—MEXICO: Coahuila—Soledad, 25 mi. s.w. of Monclova, *Palmer 856,* TYPE collection (G. US); San Lorenzo Canyon, near Saltillo, *Palmer 408* (US); Saltillo, *Palmer 120* (C, G, M, US); Sierra de Parras, *Purpus 4985,* approaches var. *argenteum* (C, M), *1878* (C, G, M, US). San Luis Potosi—between San Luis Potosi and Tampico, *Palmer 615½* (G); San Luis Potosi, *Schaffner 396* in 1879 (CA, US), *77* in 1876 (G), *Purpus 5349* (C, M); Minas de San Rafael, *Purpus 5344* (C, G).

6a. **N. Palmeri** var. **argenteum** (Brand) comb. nov. *N. argenteum* Brand, Beiträge z. Kennt. d. Hydroph. (König. Gymnas. Sorau. Beilage z. Jahresb. 9. 1911); Pflanzenr. 4^{251}: 147. 1913.

Leaves silvery-sericeous, 2–4 mm. broad, revolute; capsules 4–12-seeded; calyx-lobes not hardened in fruit.

Material seen—Ixmiquilpan, Hidalgo, *Purpus 1401,* TYPE collection (C, G, M).

The variety *argenteum* is practically identical with the species in nearly all respects, and appears to intergrade almost imperceptibly with it; therefore, *argenteum* may be but an extreme form of *Palmeri.* The fewer seeds and the less hardened and less enlarged calyx-lobes are, however, perhaps of sufficient significance to warrant separating the two varietally.

7. N. SPATHULATUM T. S. Brandegee, Zoë 5: 253. 1908; Brand, l. c. 145. Pl. 27, fig. 18.

A suffrutescent, spreading, somewhat tufted, sericeous-hirsute perennial, with heavy woody rootcrown, branches 5–15 cm. long, densely leafy, old leaves and capsules remaining at base; leaves oblong or oblong-obovate, 1.5–3.5 cm. long, 4–10 mm. broad, somewhat gray-silky, sparingly glandular beneath, sessile and somewhat clasping; flowers subsessile, borne singly or in 2's; calyx-lobes narrowly lanceolate, ca. 4.5 mm. long in flower; filaments unequally inserted ca. 1 mm. from corolla-base, somewhat flattened, the adnate bases greatly widened, their margins free as two wide scales ca. 0.7 mm. long, much widest at top; styles ca. 2 mm. long, rather weakly united about half their length; ovary covered by enlarged, hardened calyx-lobes, 40–50-seeded; seeds as in *N. Palmeri.*

Material seen—MEXICO—Cerro de Baxtla, Puebla, *Purpus 2584* TYPE collection (C, G, M, US).

Nama spathulatum is very closely related to *N. Palmeri,* but the corolla is smaller, and the filament-scales are much wider. In addition, the leaves are larger and tend to be clasping, and the styles are connate, although readily separable with dissecting needles.

8. N. STENOPHYLLUM Gray ex Hemsl. Biol. Cent.-Am. Bot. **2**: 361. 1882; Brand, l. c. 145. *Conanthus stenophyllus* (Gray) Heller, l. c. *Marilaunidium stenophyllum* (Gray) Kuntze, l. c. Pl. 27, fig. 17.

A somewhat suffrutescent, grayish-green, hirsute-hispid, very leafy perennial, the branches numerous, erect or somewhat ascending, with exfoliating, rough bark on older portions, 10–30 cm. long; leaves linear, revolute, 10–40 mm. long, 1–3 mm. broad, grayish-hispid; flowers borne in crowded, shortened, compound cymes, on pedicels 2–4 mm. long; calyx-lobes linear, 7–10 mm. long; corolla tubular-obconic, 8–10 mm. long; stamens very unequally inserted 1–3 mm. from base of corolla, filaments greatly expanded from point of adnation almost to corolla-base, the margins free, forming two broad scales that project from filament-base and make it appear that the filaments are bifid below; styles ca. 4 mm. long; ovary ca. 4 mm. long, 30–40-seeded; seeds light brown, finely though definitely alveolate.

Material seen—MEXICO: Coahuila, *Thurber 843* (G); Viesca, Coahuila, *Purpus 117* (C, G, M, P); s.w. of Parras, Coahuila, *Palmer 861,* TYPE collection (G, M, US); San Lorenzo and Parras, Coahuila, *Palmer 862,* approaching the var. *flavescens* (G, US); Chihuahua, *Pringle 120* (G, US).

8a. **N. stenophyllum** var. **flavescens** (Brandegee) comb. nov. *N. flavescens* T. S. Brandegee, Zoë **5**: 254. 1908; Brand, l. c. 158.

Plant yellow-green, sparingly strigose-hirsute and rather conspicuously glandular-mealy. Otherwise as in the species.

Material seen—MEXICO: Parras, Coahuila, *Purpus 1875,* TYPE collection (C, G, M, US); n. Zacatecas, *Lloyd & Kirkwood 143* (G); hills near Cedros, Zacatecas, *Lloyd 12* (US).

Conspicuous chiefly because of the glandular and granular pubescence. Because of the great variation in the little material available, there is some doubt in the writer's mind whether this variety would be maintainable as such in the light of evidence forthcoming from more abundant material.

8b. N. STENOPHYLLUM var. EGENUM Macbride, Contr. Gray Herb. **49**: 44. 1917. *Conanthus carnosus* Wooton, Bull. Torr. Bot. Club **25**: 262. 1898. *Andropus carnosus* (Wooton) Brand, Fedde Rep. Spec. Nov. **10**: 281. 1911 and Pflanzenr. 4^{251}: 163, fig. 32. 1913.

Plant strigose-hispid, yellowish-green when living, more woody than the species, the stems branched chiefly near the summit, 20–40 cm. long; leaves strongly revolute; seeds brown.

Material seen—UNITED STATES: White Sands, Dona Ana Co., New Mexico, *Wooton 164,* TYPE *C. carnosus* (C, M, P, US); bluffs of Delaware Creek, e. of Guadalupe Mts., Texas, *Havard 15* (G, TYPE).

The type of Macbride's variety—namely, *Havard 15*—is identical with *Wooton 164,* the type of *C. carnosus.* Brand's genus *Andropus* certainly

cannot be maintained, as the filaments are not really bifid, rather the margins of the adnate portion are very wide and form wing-like scales, a condition that is not uncommon and is identical with the condition in the species itself which Brand retained in the genus *Nama*.

9. N. SERICEUM Willd. ex Roem. & Schultes, Syst. Veg. 6: 189. 1820; Gray, Proc. Am. Acad. 5: 339. 1861; Brand, Pflanzenr. 4²⁵¹: 157, fig. 30. 1913. *Marilaunidium sericeum* (Willd.) Kuntze, 1. c. *Nama longiflora* Choisy, Mém. Soc. Phys. Genève 6: 114, pl. II, fig. 2. 1833. *N. errhina* Pav. ex Brand, 1. c. (publ. in synonymy). *Hydrolea violacea* Moc. & Sesse, ex Choisy, 1. c. (publ. in synonymy).

A suffruticose perennial, densely pilose-sericeous, the branches somewhat decumbent, 1.5–5 dm. long; leaves ovate-lanceolate to elliptic, lower surface densely sericeous, grayish, the upper surface strigose-hirsute, greenish, 1.5–8 cm. long, 0.5–2.5 cm. broad, petioles 4–10 mm. long; flowers borne in terminal or lateral, lax cymes, on pedicels ca. 5 mm. long; calyx-lobes linear-spatulate, ca. 1 cm. long, hirsute; corolla obconic-campanulate, 17–25 (15) mm. long; stamens subequally inserted about 2–4 mm. from base of corolla, filaments 10–15 mm. long, the adnate bases slightly enlarged, extending to base of corolla, their margins free; styles 10–15 mm. long; capsules 100–130-seeded; seeds brown, 0.5–0.7 mm. long, finely alveolate.

Material seen—MEXICO: without locality, *Coulter 914* and *915* (G); Tamaulipas, mts. near S. Vicente, *von Rozynski 99* (C); San Jose Pass, San Luis Potosi, *Pringle 3059* (C, G, M, P, US); Minas de San Rafael, San Luis Potosi, *Purpus 4860* (C, G, M, US); Sierra de la Mesa Ixmiquilpan, Hidalgo, *Purpus in 1905* (C).

10. N. HIRSUTUM Mart. & Gal. Bull. Acad. Sci. Brux. 12²: 277. 1845; Gray, Proc. Am. Acad. 5: 339. 1861; Brand, 1. c. 148. *Marilaunidium hirsutum* (Mart. & Gal.) Kuntze, 1. c. Pl. 27, fig. 23.

An ascending, slender, strigose-hirsute perennial, the old branches somewhat woody, trailing or erect, young branches herbaceous, more erect, 1–8 dm. long; leaves ovate, oblong-ovate, or elliptic-lanceolate, 1.5–8 cm. long, 0.5–3 cm. broad, with slender petioles 1/5–1/3 length of blade; flowers borne in scattered, lax, 6–10-flowered compound cymes; calyx-lobes narrowly oblanceolate-spatulate, 5–6 mm. long (7–8 mm. long in fruit); corolla obconic, 7–10 mm. long; stamens unequally inserted 1–2 mm. from corolla-base, free filaments flattened, much widened from about 1 mm. above point of insertion to that point, the adnate bases about same width, with free margins; styles 4–5 mm. long; capsules 4–12-seeded; seeds brown, ca. 0.7 mm. long, mealy-granular.

Material seen—MEXICO: Oaxaca—without locality, "ex Martens & Galeota" fragment of TYPE (G); Hacienda de San Luis, *Conzatti 191* (G); Cerro de San Felipe, *Conzatti 206* (G), *Nelson 1182* (US); Sierra de San Felipe, *Pringle 4791* (C, G, M, P, US), *Smith 818* (M, US); Tres Cruces, *Reko 3341* (US); Cañada de San Gabriel-Erta, *Conzatti & Gonzales 293* (G).

11. N. PURPUSII T. S. Brandegee, Univ. Cal. Pub. Bot. 4: 186. 1911; Brand, 1. c. 159. Pl. 27, fig. 32.

An erect, grayish, densely hirsute-hispid, somewhat suffrutescent perennial, branches very leafy, 10–25 cm. long; leaves linear-spatulate or linear,

15–25 mm. long, 2–4 mm. broad, very strongly revolute; flowers on small short branches along the stem; sepals linear, 5–6 mm. long; corolla tubular-campanulate, 10–12 mm. long; stamens very unequally inserted, free filaments about 1 mm. long, rather thick, the adnate bases from 3 to 6 times as long as the free portion, much widened and thickened, the free margins rather wide, extending to base of corolla; styles 3–4 mm. long; ovary with ca. 80 ovules; mature seeds not seen.

Material seen—Mexico: Movano, Coahuila, *Purpus 4562* (C, Type).

12. N. origanifolium H. B. K. Nov. Gen. et Spec. Pl. 3: 130, pl. 218. 1818; Choisy, Mém. Soc. Phys. Genève 6: 113. 1833; Gray, Proc. Am. Acad. 5: 337. 1861, in small part. 8: 284. 1870; Brand, l. c. 149. *N. origanifolium* subsp. *eu-origanifolium* Brand, l. c. 150. *N. origanifolium* subsp. *eu-origanifolium* var. *genuina* and var. *pedunculatum* Brand, l. c. *N. subincana* Willd. ex Roem. & Schult. Syst. Veg. 6: 189. 1820. *Hydrolea tenella* Moc. & Sesse ex Choisy, l. c. *Conanthus origanifolius* (H. B. K.) Heller, l. c. *Marilaunidium origanifolium* (H. B. K.) Kuntze, l. c. Pl. 27, fig. 21.

A grayish, velvety to strigose-villous and glandular perennial, the base woody, branches very slender, trailing or scandent, 5–30 cm. long; leaves linear to narrowly spatulate or obovate, mostly about 7 mm. long, 1.5–4 mm. broad, revolute, the veins prominent, petioles 1–2 mm. long; flowers 1–3 at the nodes or in reduced cymes, on pedicels 3–15 mm. long; calyx-lobes linear-spatulate, ca. 3 mm. long, corolla tubular, 4–6 mm. long; stamens equally inserted about ½ mm. from base of corolla, the free filaments slightly flattened and widened gradually to point of insertion, without free margins below; styles 1.5–2 mm. long; capsules 35–60-seeded; seeds very dark brown, ca. 0.4 mm. long, minutely alveolate, the pits in more or less regular rows.

Material seen—Mexico: San Luis Potosi—without locality, *Schaffner 361* (CA, US); reg. of San Luis Potosi, *Parry & Palmer 612* (G and M in part, US); San Miguelito, *Schaffner 732* in 1876 (G). Hidalgo—Ixmiquilpan, *Purpus 480* (C, G, M, P). Puebla—Cerro de Gentile, *Purpus 2585,* Type of var. *pedunculatum* (C); Cerro de Chicamole, *Purpus 3909* (C).

12a. **N. origanifolium** var. **rupicolum** (Bonpl. ex Choisy) comb. nov. *N. rupicola* Bonpl. ex Choisy, l. c. 114; Gray ex Hemsl. Biol. Cent.-Am. Bot. 2: 363. 1882, in part. *Marilaunidium rupicolum* (Bonpl.) Kuntze, l. c. *N. origanifolium* subsp. *rupicolum* Brand, l. c. 150. *N. origanifolium* subsp. *rupicolum* var. *eu-rupicolum* Brand, l. c.

Branches as much as 4 dm. long; leaves ovate to oblong, 1–2.5 cm. long, 4–15 mm. broad, petioles slender, grayish-silky-villous; flowers in lax cymes on slender peduncles and pedicels; corolla nearly 6 mm. long.

Material seen—Mexico: "Nama rupicolum herb. Pavon, Nueva Espana" from herb. Boissier (G, fragment of Type); Sierra Madre, n. Mexico, *Seeman 2083* (G). Nuevo Leon: Bishop's Hill, Monterey, *Gregg 182* (M). San Luis Potosi: San Luis Potosi, *Purpus* in 1905 (C); reg. of San Luis Potosi, *Parry & Palmer 612* (G, M in part only, but not US); Santa Maria del Rio, *Palmer 158* (US). Queretaro—near Queretaro, *Rose & Rose 11193* (US). Jalisco: near Guadalajara, *Pringle 2977* (G). Morelos: near Cuernavaca, *Barnes & Land 470* (US), *Bilimik 392* (G), *Bourgeau 1263* (G), *Lemmon & Lemmon 225* (C), *Pringle 6160* (C, G, M, US), *11039* (G, M, US).

This variety seems almost to merit specific recognition, due chiefly to the more lax character of the plant and to the much larger and more tender leaves; however, because of the fact that the flower and seed characters of the two are the same, and because of the fact that they intergrade, they can best be treated as variety and species.

13. **N. xylopodum** (Woot. & Standl.) comb. nov. *Marilaunidium xylopodum* Woot. & Standl. Contr. U. S. Nat. Herb. **16**: 162. 1913. Pl. 27, fig. 25.

A tufted, strigose-hispid perennial, the branches numerous, slender, simple, leafy, 5–10 cm. long, arising from thick woody crown; leaves elliptic-oblanceolate, about 1 cm. long, 2–4 mm. broad, hispid, somewhat glandular beneath, slightly revolute, nearly sessile; flowers borne singly or in few-flowered, lax, terminal cymes, on pedicels 1–2 mm. long; calyx-lobes linear, 4–5 mm. long; corolla tubular-obconic, 6–8 mm. long; stamens unequally inserted 0.5–1.5 mm. from base of corolla, filaments somewhat flattened, greatly widened at point of adnation, the free margins wide and toothed at top, the scales narrowing toward base, extending nearly to corolla-base; styles ca. 2 mm. long; ovary about 150-ovuled, but mature capsules apparently with only about 40 seeds; seeds yellow, ellipsoid, 0.5 mm. long, reticulate.

Material seen—without data, in the Reverchon herbarium (M). UNITED STATES: Queen, Eddy Co., New Mexico, July 31, 1909, *Wooton* (US, TYPE); Guadalupe Mts., Culbertson Co., Texas, *Havard 15½* (G), *Moore & Steyermark 3562* (C, M).

This species has apparently been mistaken for *N. origanifolium* by most workers, due undoubtedly to the similarity in habit of the two species. *Nama xylopodum* can be readily told from the Mexican species, however, by the differences in stamen character.

14. **N. ROTUNDIFOLIUM** (Gray) Macbridge, Contr. Gray Herb. **49**: 44. 1917. *N. rupicolum* Bonpl. var. *rotundifolium* Gray, Hemsl. Biol. Cent.-Am. Bot. **2**: 363. 1882. *N. origanifolium* H. B. K. subsp. *rupicolum* (Bonpl.) Brand var. *rotundifolium* (Gray) Brand, l. c. 150. Pl. 27, fig. 9.

A delicate, slender, rather densely villous (viscid?) perennial, the branches weak, ascending, 10–20 (30) cm. long; leaves rotund, broadly ovate, or oblong-ovate to obovate, 1–3 cm. long, 3–15 mm. broad, very thin and delicate, on very slender petioles 3–10 mm. long; flowers borne singly or in 2's or 3's on very slender filiform pedicels 1–3 cm. long; calyx-lobes linear, 4–6 mm. long; corolla tubular, 7–9 mm. long; stamens unequally inserted ca. 1 mm. from base of corolla, free portion of filament filiform, adnate portion widened, the free margins widest somewhat below point of insertion, running nearly to base of corolla; styles ca. 2 mm. long; capsules ca. 150-seeded; seeds brown, ca. 0.3 mm. long, very finely and minutely transversely corrugated.

Material seen—MEXICO: Monclova, Coahuila, *Palmer 983*, TYPE collection (G, US); Coahuila? *Palmer 982* (US one sheet, but not G); Monterey, Nuevo Leon, *Palmer 984* (G, US).

The resemblance of this plant to *N. origanifolium* is entirely superficial. The stamen differences, and the difference in the markings of the seeds show that the two species are entirely distinct.

15. **N. SERPYLLOIDES** Gray ex Hemsl. Biol. Cent. Am. Bot. **2**: 363. 1882;

Brand, l. c. 149. *Marilaunidium serpylloides* (Gray) Kuntze, l. c. Pl. 27, fig. 3.

A diffusely branched, hirtellous perennial, the old branches near the base somewhat woody, young branches filiform, exceedingly numerous, 10–40 cm. long; leaves thin and tender, obovate or obovate-spatulate, 7–15 mm. long, 2–7 mm. broad, with short winged petiole, prevailing opposite; flowers borne singly or in 2's on filiform, curved pedicels ca. 1 cm. long; calyx-lobes linear, 2–3 mm. long; corolla narrowly obconic, 5–6 mm. long; stamens unequally inserted ca. 1 mm. above corolla-base, the free filaments filiform, 1–1.5 mm. long, the adnate bases slightly widened, with very narrow freemargins just below point of insertion; styles ca. 1.5 mm. long; capsules apparently few-seeded; seeds brown, ca. 0.3 mm. long, finely alveolate.

Material seen—MEXICO: Coahuila, without locality, *Palmer 985* (US); Monclova, Coahuila, *Palmer 982*, TYPE collection (G, US one sheet only).

16. N. BIFLORUM Choisy, DC. Prodr. **10**: 183. 1846; Gray, Proc. Am. Acad. **5**: 337. 1861; Brand, l. c. 146. *Marilaunidium biflorum* (Choisy) Kuntze, l. c. *N. subpetiolare* Gray ex Hemsl. Biol. Cent.-Am. Bot. **2**: 365. 1882; Brand, l. c. 148. *Marilaunidium subpetiolare* (Gray) Kuntze, l. c. *N. jamaicense* L. var. *longipedicellatum* Loesener ex Brand, l. c. 146 (publ. in synonymy). Pl. 27, fig. 2.

An ascending, leafy, sericeous or villous-hirsute annual (biennial?), the branches 10–40 cm. long; leaves grayish, strigose to villous, ovate or oblong to obovate-spatulate, 2–6 cm. long, 0.5–2.5 cm. broad, the tapering petioles winged, ¼ as long to as long as the blades, decurrent on the stem; flowers borne in 2's or 3's (5's) in the axils, on long filiform peduncles and pedicels 1–4 cm. long; calyx-lobes linear (sometimes enlarged slightly above middle) 5–7 mm. long; corolla narrowly tubular-obconic, 7–10 mm. long; filaments unequally inserted ca. 1.5 mm. from corolla-base, terete above, but flattened and much expanded about 1 mm. above insertion, the adnate bases expanded and with free margins nearly to base of corolla; styles ca. 4 mm. long, usually united ⅓–½ their length, rarely distinct; capsules 40–100-seeded; seeds brown, reticulate-alveolate, the pits in rather definite longitudinal rows.

Material seen—MEXICO: Nuevo Leon: Monterey, *Palmer 985*, TYPE collection of *N. subpetiolare* (G, US one sheet only), *Palmer 984* (US), *Pringle 2206* (G). San Luis Potosi: Rio Verde, *Palmer 23* (M, US, approaching *N. jamaicense*); San Luis Potosi to Tampico, *Palmer 702½* (G, US, approaching *N. jamaicense*). Tamaulipas: near Victoria, *Runyon 928* and *962* (US), *Palmer 524* (US); between Victoria and Tula, *Berlandier 2200=780* (G, TYPE collection of *N. biflorum*, cited by Choisy as no. *220*); mts. s. of Victoria, *Runyon 753* (US); Cerro Zamora, vic. of El Milagro, *Bartlett 11135* (US); La Tamaulipèca, vic. of San Miguel, *Bartlett 10706* (US); vic. of Tampico, *Palmer 2* (CA, M, US, approaching *N. jamaicense*). Vera Cruz: Vera Cruz, *Seler 3704* (G, intermediate between *N. biflorum* and *N. jamaicense*).

The extreme form of this species is very different from the bulk of the material of *N. jamaicense*, but the two species intergrade by almost imperceptible stages; it therefore seems possible that *biflorum* is only a variety of the latter. However, the aggregate of characters, namely, pubescence, smaller leaves, longer pedicels, larger corolla, calyx not coherent to ovary, longer styles that are more frequently united, and darker seeds, makes it seem best

to conserve both entities as species. *Nama subpetiolare* is not maintainable as an entity, the type being but an extreme form of *biflorum;* the corolla characters, pubescence, seeds, connate styles, calyx-lobes, and winged-petioles are those of *biflorum,* the only significant difference being that the leaves are thicker than is the usual condition. Brand did not see any material of *N. subpetiolare* and misinterpreted the species.

17. N HAVARDII Gray, Proc. Am. Acad. **20**: 304. 1885; Brand, l. c. 160. *Conanthus Havardii* (Gray), Heller, l. c.

An erect, leafy, cinereous-sericeous-villous annual (perennial?), little if at all woody at base, 1.5–4 dm. tall; leaves oblong-elliptic to obovate, 2–4 cm. long, 4–13 mm. broad, narrowed to slender petioles; flowers borne in numerous, few-flowered, compound, lax cymes, on pedicels ca. 2 mm. long; calyx-lobes narrowly linear-spatulate, 6–9 mm. long; corolla tubular-campanulate, 9–11 mm. long; stamens unequally inserted 3–4 mm. from base of corolla, the free filaments ca. 1.5 mm. long, terete, adnate portion thickened and much expanded, the free margins quite wide, running almost to base of corolla (as in *N. Purpusii*); styles ca. 4 mm. long; capsules 40–60-seeded; seeds brown, ovoid-ellipsoid, ca. 0.6 mm. long, shallowly pitted and minutely alveolate, markings much the same as in *N. dichotomum.*

Material seen—UNITED STATES: Texas—Brewster County, Hot Springs, *Jones 25737* (P); 3 mi. e. of Study Butte, *Moore & Steyermark 3248* (C, M); bank of Tornillo Creek, *Havard 95½* (G, TYPE); Hot Springs, Rio Grande near mouth of Tornillo Creek, *Cory* in 1928 (G).

Because of the fact that *N. Havardii* has been reported as a perennial the writer is including it in the perennial species, although, judging from the appearance of the plants collected by Moore and Steyermark, it seems more probable that it is a very robust annual.

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EXPLANATION OF PLATE 27

Fig. 1–14. Seeds of *Nama* and *Eriodictyon* spp., showing characteristic markings (shape of seeds usually variable).

Fig. 1. *N. dichotomum.* × 50. Fig. 2. *N. biflorum.* × 50. Fig. 3. *N. serpylloides.* × 80. Fig. 4. *N. Lobbii.* × 15. Fig. 5. *N. depressum.* × 40. Fig. 6. *N. stenocarpum.* × 80. Fig. 7. *N. demissum.* × 40. Fig. 8. *N. pusillum.* × 60. Fig. 9. *N. rotundifolium.* × 80. Fig. 10. *N. undulatum.* × 60. Fig. 11. *N. Rothrockii.* × 15. Fig. 12. *E. Parryi.* × 15. Fig. 13. *N. torynophyllum.* × 60. Fig. 14. *N. densum.* × 40.

Fig. 15–32. Portions of corollas of various species of *Nama* opened to show nature of filaments. × 5. Arrow indicates point of adnation of filaments with corolla.

Fig. 15. *N. undulatum.* Fig. 16. *N. stenocarpum.* Fig. 17. *N. stenophyllum.* Fig. 18. *N. spathulatum.* Fig. 19. *N. jamaicense.* Fig. 20. *N. Palmeri.* Fig. 21. *N. origanifolium.* Fig. 22. *N. torynophyllum.* Fig. 23. *N. hirsutum.* Fig. 24. *N. sandwicense.* Fig. 25. *N. xylopodum.* Fig. 26. *N. pusillum.* Fig. 27. *N. Stevensii.* Fig. 28. *N. prostratum.* Fig. 29. *N. dichotomum.* Fig. 30. *N. Schaffneri.* Fig. 31. *N. depressum.* Fig. 32. *N. Purpusii.*
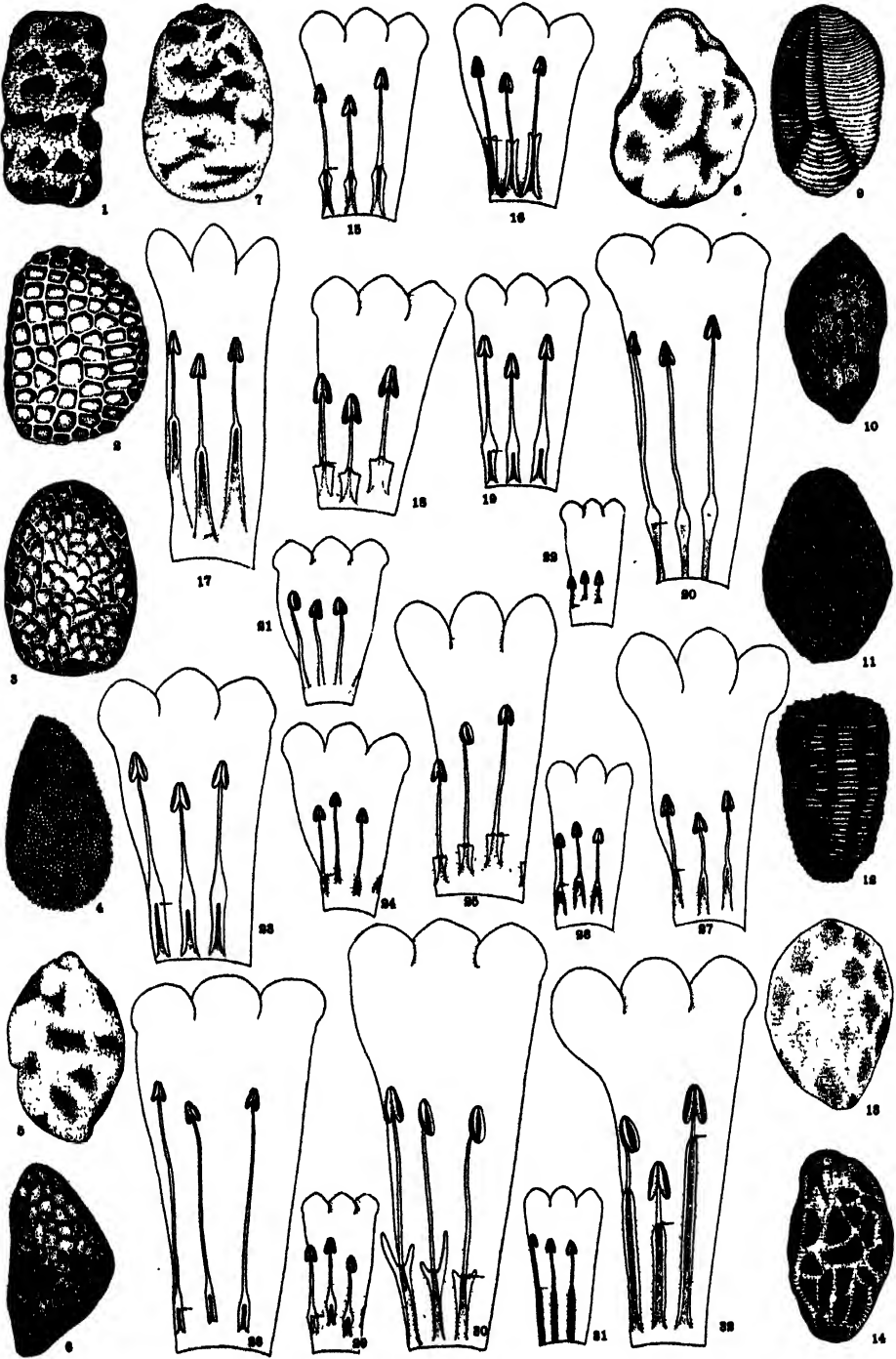
NEUTRAL RED STAINING IN THE PROTONEMA OF
POLYTRICHUM COMMUNE

T. Elliot Weier

Since the pioneer work of Pfeffer it has been known that the large plant vacuole forms through the fusion of numerous small vacuoles present in the meristematic tissue of roots and shoots. Most of our present knowledge concerning the plant vacuole was accumulated during the years immediately following this work. These facts have been summarized by Crié. In recent years numerous new facts have been added to our knowledge of the vacuole, the most significant of which have been the reports by Dangeard (1923) and Guilliermond (1930) that the vacuole takes a network ,formation during its transition from the stage of numerous small vacuoles of meristematic tissue to the single large vacuole of fully differentiated tissue. Many of these investigations have been carried out with neutral red staining on living cells. Dangeard's work, in particular, is interesting, although it is somewhat marred by the fact that the staining was entirely confined to regions approximating a wound. It seems entirely possible that injury may have in some manner affected the staining reaction.

Parat and Painlevé (1924), working with neutral red on the salivary gland of the chironomus larva, observed that certain granules accumulate the stain. These granules swell, and fuse to form an irregular mass that is ejected from the cell. From the apparent similarity of this phenomenon occurring in highly specialized animal tissue to the formation of vacuoles in embryonic plant tissues, they drew the generalization that the two phenomena were homologous. Furthermore, since their work was carried out on gland cells, they further infer that the secrotogenous region, the Golgi zone, is homologous with the plant vacuole. Upon the basis of this work the generalization that the neutral red staining structures are homologous in both plants and animals has been extended and further developed by a large number of zoologists.

There is, indeed, much to be said in favor of this concept. In addition to the apparent similarity of vital staining, mention may be made of the structure of the plant vacuole after osmification (Guilliermond, 1930) and the similarity found to exist between the negative image of the Golgi zone and the appearance of plant vacuoles in elongating tissue (Bensley, 1910). It appears to the writer that the crux of much of the present confusion concerning the nature of the Golgi zone lies in the validity of this generalization. In other words, if, as Cowdry, Bensley, Guilliermond, Parat, and others claim,

the Golgi apparatus is comparable to the plant vacuole, the inevitable conclusion that the Golgi zone is a watery "dead" vacuole must follow. That there are, however, numerous serious objections to considering the Golgi apparatus and the plant vacuole to be homologous have been brought out by the writer in previous reports (Weier, 1931, 1932a, 1932b, 1932c).

These previous reports have been concerned with fixed and stained preparations. In this work I had hoped to be able to compare directly the staining of the salivary gland of the chironomus larva and a type of neutral red staining of plant tissue observed some years ago which apparently corresponded in some degree with Parat's account of the neutral red staining of the salivary gland cells. The larvae were easily obtained in great numbers on the campus of St. Lawrence University.[1] They were brought into the laboratory and allowed to build their cases. I was unable, however, to duplicate Parat's experiment. Granules in the salivary gland did stain, frequently taking an irregular form, but never, although numerous variations of the technique were tried, did these granules flow together and pass out of the cell. Parat reports that his experiments were carried out under very precise conditions, but he neglects to state just what these conditions are. It is very possible that I did not obtain the exact conditions necessary for the successful operation of the experiment. In spite of these negative results, together with similar failures encountered by Krjukowa (1929) and Beams and Goldsmith (1930), I have at present no doubt of the essential facts of the development of the neutral red granules in the salivary gland of the chironomus larva. It does seem, however, that the phenomenon must occur under rather special conditions which can hardly be comparable to the formation of plant vacuoles in the regions of elongation. The similarity of plant vacuole and the neutral red granules of the chironomus salivary gland, from a viewpoint of cell physiology, is rather vague, so much so that it seems to the writer that with a little serious consideration the essential dissimilarity of the two phenomena must at once be apparent.

Due to my inability to duplicate Parat's experiments, it was not possible to make a direct comparison of the neutral red staining in plant and animal forms. The report then will be confined to a description of neutral red staining in *Polytrichum commune* and its possible bearing on the neutral red staining of animal cells. It will be seen that in two important respects the type of staining in the protonema is comparable to the vital staining of the larva gland cells. First, in both cases the cells are not embryonic, and second, the large mass formed from the accumulation of the small neutral red granules is ejected from the living cytoplasm.

MATERIALS AND METHODS

Protonema of *Polytrichum commune* was grown under sterile conditions on nutrient gelatine and on filter paper in culture solution. When ready

[1] The larvae were kindly identified for me by Professor J. L. Buys, entomologist at St. Lawrence University.

for observation the protonema was carefully mounted on a glass slide either in culture solution or in tap water. The preparations were then ringed with melted vaseline. In either medium the protonema remained alive and normal for two weeks or longer. The neutral reds were kindly furnished me by Dr. H. J. Conn, of the Stain Commission. They bore the marks NX 1, NX 3, NX 4, and neutral red iodide. The NX 3 sample was considerably less toxic than the others. The stain was dissolved in absolute alcohol. Drops of the stock solution were placed by means of a micropipette on the cover glass and allowed to dry. The cover slip was then inverted over the drop of water containing the protonema. In this manner the neutral red was brought immediately into intimate contact with all of the cells of the filament. The dilution most frequently used was approximately 1 : 5000.

OBSERVATIONS

Normal unstained protonemal cell

In the unstained cell of the protonema the chloroplasts are irregularly hexagonal in shape. They are usually flat, with their broad surfaces forming a mosaic around the outer edge of the protoplast (fig. 1). Numerous small, highly refractive granules are present in the cytoplasm between the chloroplasts. These vary somewhat in size, although they do not apparently grade down to the limit of visibility. Upon rare occasions I have observed cells in which these granules were not visible. It seems that after 48 hours or more the granules become larger and less numerous, suggesting that some of them may have flowed together. It is not possible in the living cell to differentiate more than one kind of granule, although, as will be noted later, they do not all stain with neutral red. No filamentous structures are present in the cytoplasm. Experiments with Janus green all yielded negative results. The granules are preserved by mitochondrial techniques and stain with iron alum-haematoxylin. However, since their nature and function are quite unknown, it seems wisest not to class them as mitochondria unless one means by mitochondria merely a granule of unknown nature and function. In many quarters, however, the term mitochondria is accepted as a definite solution of cytoplasmic problems, and since as yet nothing is known of the nature and function of these granules, it is perhaps best not to characterize them as mitochondria. They will be referred to merely as granules. A large sap vacuole fills the central portion of the cell, while the nucleus is difficult to observe in the living cell.

The large amount of architectural detail in the protonemal cell renders it extremely valuable for vital studies in that any change in relations between vacuole, cytoplasm, chloroplasts, and granules is easily noted. In the animal cell it is conceivable that much change may occur in cellular structure without any visible evidence.

Neutral red staining

The cell accumulates the dye slowly, so that, upon examining a fresh neutral red mount immediately, the cells of the filament contain no stain and appear in all respects similar to the unstained preparations. Slowly some of the granules accumulate the stain (fig. 2). Others remain perfectly colorless. The penetration of the stain is more rapid in the cells located at the ends of the filaments. Cells in the young leaves seldom, if ever, stain when they are in a healthy, normal condition. Protonemal cells located in a mass of tangled filaments never stain. This can hardly be due to the failure of the dye to reach the filaments, since the dry stain on the cover glass must come into intimate contact with all of the moss cells.

The granules which accumulate the stain become brilliantly red and slowly swell. In doing so, they come into contact with each other. They may remain in contact as distinct globules for some time. Finally, through some sudden change in surface tension two of them will fuse together; then others fuse until one, two, or a dozen rather large, deeply stained globules are present in the cytoplasm of the cell (fig. 3, 6, 12). Early in the process of staining, the vacuole, which in the normal unstained cell shows no definite line of demarcation between the sap and cytoplasm, contracts, at the same time bringing into prominence a distinct membrane separating the vacuolar sap from the cytoplasm. The ends of the vacuole are now rounded and have been drawn some distance from the ends of the cell (fig. 3a, 8a). One or more neutral red globules may be seen in the cytoplasm between the vacuole and the end of the cell (fig. 3a). They sooner or later come into contact with the limiting membrane of the vacuole, where they form a large, red, irregular mass (fig. 4g). The stain now diffuses rather rapidly into the sap vacuole; the neutral red globule meanwhile may continue to increase in size and to accumulate stain. In addition to this mode of conduction of the stain to the sap vacuole, some dye reaches the vacuole by direct diffusion through the cytoplasm.

Concurrent with the shrinkage of the vacuole from the ends of the cell it increases in girth so as to completely fill the diameter of the cell (fig. 13). The result of this change in the shape of the vacuole is marked by a change in the distribution of the cytoplasm and the chloroplasts (fig. 13). The chloroplasts very soon lose their characteristic shape (cf. fig. 1 and 7) and clump together at the ends of the sap vacuoles (fig. 13). As the staining deepens, they become more and more distorted in shape until they finally come to form merely an amorphous green mass within the cell (fig. 10).

At this juncture a change in surface tension apparently occurs, for the limiting membrane between vacuole and neutral red globule suddenly disappears (fig. 5, 9). The vacuole flows slowly into the space occupied by the neutral red globule (fig. 10ag). The vacuole slowly extends its way into the mass of chloroplasts (fig. 11ag). Its slow movement suggests a rather viscous fluid. The entire cell may eventually come to be quite red. This process may take from six to twenty-four hours for its completion.

Such changes in the structure of the cell have now taken place as to cause its death. In some preparations death occurs before the disappearance of the membrane limiting the extent of the vacuole. In either case the filament usually retains the red color of the dye for a week or ten days, even after the color of the chloroplasts has faded. In preparations stained with weaker dilutions of the dye, the color may fade slowly.

It seems that in certain cells one neutral red globule may rapidly increase in size until it eventually comes not only to fill over one-half of the cell but practically to displace the ordinary sap vacuole. In instances of this kind there will usually be present in the cell a number of smaller neutral red globules (fig. 6). In this figure two small red globules are located in the end of the cell. Occupying a goodly portion of the cell is the single large red globule. Closely appressed to it on the bottom side is a small red globule being absorbed by the larger globule. Further down is another red globule, and at the bottom of the cell one may observe the mass of distorted chloroplasts. Usually the separate globules are connected by a very delicate red thread. The large red globule slowly enlarges, so that it will eventually come into contact with and engulf the smaller globules. The stain finally comes to fill much of the cell (fig. 7).

Still another variation of the staining may occur. The small granules accumulate the stain, swell, and fuse, so that the cell finally comes to contain in its cytoplasm from ten to a dozen or more rather large globules. Figure 12 shows a portion of such a cell. Changes in surface tension now cause the limiting membranes of these globules to break up, and the dye in the globules suddenly but slowly diffuses into the vacuole and cytoplasm. Instead, however, of the cell taking a deep red stain, the color becomes fainter and soon completely disappears. In this instance the cytoplasm, vacuole, and plastids have maintained their normal relations, so that after the disappearance of the color the cell is to all appearances alive and normal.

Staining with very weak dilutions of the stain give rather interesting results. When using a dilution of about 1 in 30,000, only a few cells accumulate the coloring material. Usually the cells stain as described above, but in many cases the granules will accumulate the stain but will neither swell nor fuse. They retain the stain for some little time and then suddenly become perfectly colorless. During this time there has been no change in the relation between cytoplasm, plastid, and sap vacuole.

The essential points of the coloration of *Polytrichum commune* protonema by neutral red appear to be as follows: (1) The staining is taking place in what apparently are not embryonic cells, even though they may later give rise to cells of embryonic nature. (2) The neutral red first stains granules located in the cytoplasm. (3) These granules accumulate more stain, swelling as they do so, and finally fuse. (4) Although the stain may diffuse directly into the sap vacuole, it appears that in many instances it also reaches the sap vacuole by the way of these neutral red granules. (5) In

most instances this phenomenon is pathologic.[2]   (6) In some instances, particularly when weak dilutions of the stain are used, the dye is apparently rendered harmless and colorless by the granules or by the granules and the cytoplasm.

<div align="center">DISCUSSION</div>

It would be interesting to try to follow the physical changes taking place in the cell during the formation of the large neutral red globules, their passage into the sap vacuole, and the discoloring of the vacuole.   The pressure of teaching duties at St. Lawrence University during my stay there made this impossible, and now the economic situation has separated me from microscope and protonema, so that any discussion of this nature must be postponed.

From the point of view of the vacuolar-Golgi discussion some very interesting things are brought out by these experiments. First, neutral red granules in differentiated plant cells hydrate, fuse, and are ejected from the living cytoplasm into the sap vacuole.   Second, this phenomenon is essentially different from the enlarging and coalescing of the small embryonic vacuoles in elongating plant tissues.   Third, the coalescence of the granules in the differentiated cells of *Polytrichum commune* either is or brings about a pathological condition from which the cell dies.   Fourth, this phenomenon is more similar to that described by Parat and Painlevé in that (*a*) it occurs in non-embryonic tissues and (*b*) the neutral red substance is finally ejected from the living cytoplasm of the cell.   Fifth, in view of this similarity due consideration should be given to its comparisons between plant and animal tissue.

In view of the similarities between neutral red staining in some animal tissue and that described in this work, I should like to suggest the possibility of a basic resemblance between neutral red staining in animal cells and that described here.   Pfeffer many years ago postulated a very plausible explanation for this very phenomenon in plants, and there are certain indications that his explanation will also fit neutral red staining in animal cells.   Pfeffer found that certain granules in root hairs of *Trianea bogolensis* stained with Bismarck brown.   These granules were finally ejected into the vacuole.   This, according to Pfeffer, is illustrative of a normal protective mechanism whereby the cell is enabled to adapt itself to certain slightly toxic compounds which may at times be found in its environment.   The toxic compound is held to diffuse into the cytoplasm, where it combines with granules or bits of cytoplasm in such a manner that its toxic properties as well as its ability to diffuse through the cytoplasm are lost.   In this harmless form it is ejected from the living cell material into the sap vacuole.

This is apparently what takes place when dilute solutions of neutral red are used as a mounting medium for the moss protonema.   The stain diffuses into the cell and accumulates in certain granules.   Depending upon the concentration of the dye, the granules may or may not fuse.   When the

[2] Politzer (Biochem. Zeit. **151**) has recently described the toxic effect of neutral red on cell division in animal cells.

concentration is such that the granules do not fuse, they can apparently render the dye innocuous, for they soon become colorless and to all appearances the cell continues its normal life. With more stain, however, the granules fuse and are ejected into the vacuole, where, after a very short interval, the color disappears and the cell appears quite normal. In a strong concentration of the dye the cell makes an attempt to adapt itself to the surroundings but is prevented from doing so because the amount of stain is too great to be rendered innocuous by the cell.   .

May not this be exactly what happens in the case of the neutral red staining of the salivary gland of the chironomus larva? Certain granules accumulate the stain, they flow together, and are eventually forced out of the cell. But why are these neutral red granules so apparently in so many instances associated with the Golgi zone? It seems to the writer that the Golgi zone in its rôle of elaborator of secretions may be part of the normal mechanism for the ejection of material out of the animal cell. Since this is the case, the normal path for the neutral red granules from the interior of the cell to the exterior would be by way of the Golgi zone.[3]

Further evidence for the concept of a normal resistance mechanism in the formation and flowing together of these neutral red granules is found in the fact that vacuoles form under the influences of such things as bacteria, albumen, and toxic substances in the culture media (Lewis, 1920), as well as under the influence of caffeine and ammonia (Scarth, 1926). In each case it is perfectly conceivable that the foreign substance may have united with something within the cell, thus being rendered non-toxic to the cell. The cell might then be expected to eliminate the now harmless compound, either passing it directly out of the cell or by first digesting it to less complex, more easily diffusible, non-toxic substances. It is interesting in this connection to note that Parat and Painlevé, who alone have succeeded in observing the flowing together of the neutral red granules in the salivary gland cell of the chironomus larva, used in many instances larvae which were parasitized.

SUMMARY

1. In the protonema of *Polytrichum comunne* certain granules located in the cytoplasm accumulate neutral red, swell, fuse, and become incorporated in the sap vacuole.

[3] In a recent paper Beams and King (Jour. Morphol. 53: 1–22) have suggested that the neutral red granules may enter the cell by way of the Golgi zone. This, of course, is possible; and in any event the presence of the granules in the Golgi zone is accounted for. It seems more plausible to the writer that the dilute neutral red should diffuse into the cell from the surrounding lymph, that it should then accumulate in the granules within the cell, and finally be ejected from the cell through the Golgi zone. According to this scheme, the neutral red would follow what is probably the normal mode of entry of extracellular materials into the cell, as well as what is probably the normal mode of ejection of certain cellular substances out of the cell. According to the scheme presented by Beams and King, the probable normal mode of transfer of materials in and out of the cell would have to be reversed.

2. When relatively concentrated dilutions of the stain, 1 : 5000, are used, this phenomenon is accompanied by a change in the general architecture of the cell of a pathological nature from which the cell does not recover.

3. Under certain other conditions the mode of passage of neutral red from globule to vacuole is such that the neutral red rapidly decolorizes, the pathological condition does not arise, and the cell to all appearances continues its normal existence.

4. When very dilute solutions, 1 : 30,000, of the neutral red are used, the granules do not flow together. They accumulate the stain, remain red for some time, and gradually decolorize. A pathological condition is not brought about.

5. Staining of this type corresponds better with neutral red staining of certain animal cells than does the staining of the forming vacuoles in elongating roots and shoots.

6. It is suggested that this neutral red staining of the moss cells may be a normal protective mechanism whereby the cell is enabled to adapt itself to slightly toxic surroundings by rendering the toxic substance non-toxic by reason of its union with particles of protoplasm or of granules formed by the cytoplasm.

7. It is further suggested that the neutral red staining of animal cells may be a comparable phenomenon.

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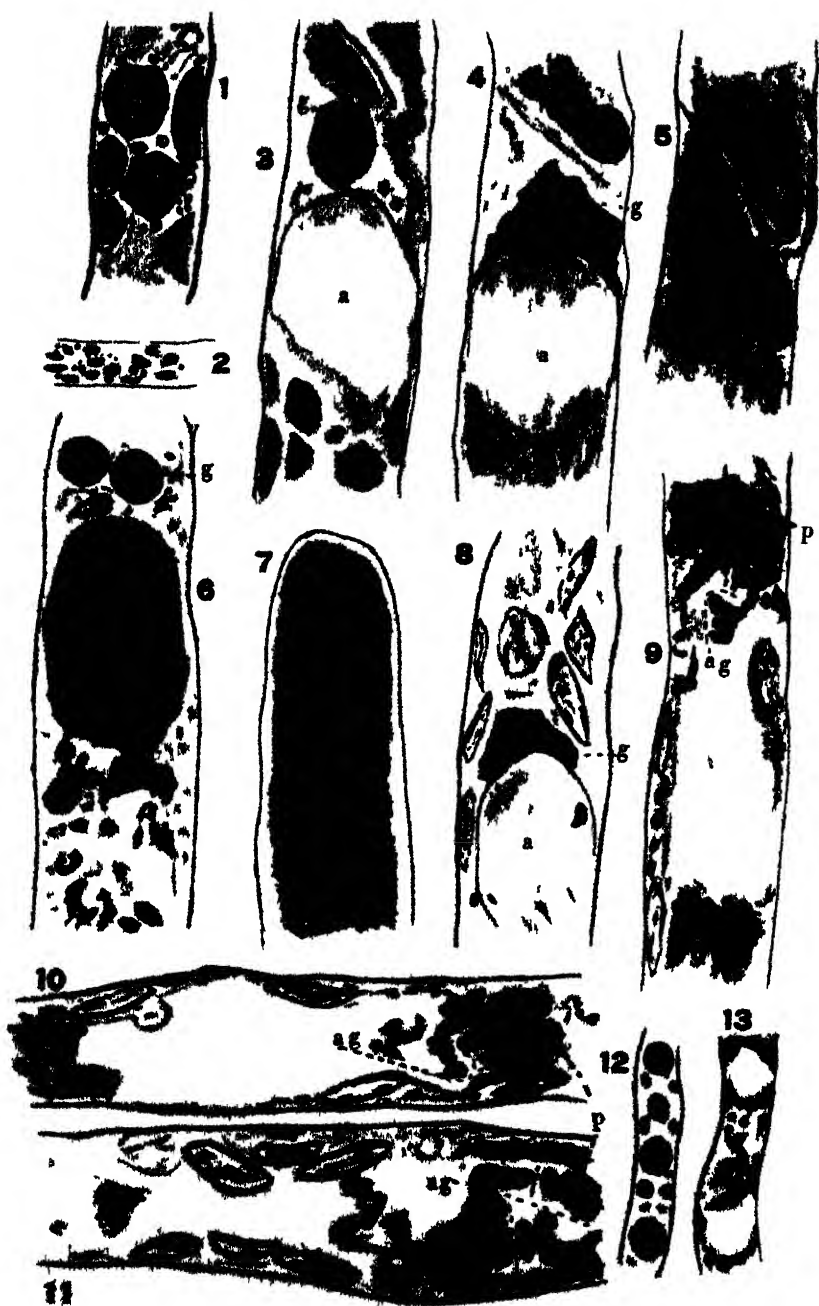
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EXPLANATION OF PLATE 28

Figures 1 and 3 to 11, inclusive, were drawn from photographs made with a Leitz microphoto attachment on Agfa process panchromatic plates. No filter was used. Figures 2, 12 and 13 were drawn from photographs made with a small Eimer and Amend microphoto attachment on Kodak roll film. No filter was used.

Fig. 1. Section of an unstained living cell of *Polytrichum commune*. The chloroplasts, roughly hexagonal in shape, form a mosaic just under the cell wall. Granules may be observed in the cytoplasm between the chloroplasts.

Fig. 2. A section of a cell which has just been placed in the stain. Some of the granules have stained and show here as blackened granules. The normal distribution of the chloroplasts has changed slightly.

Fig. 3. Neutral red staining. The chloroplasts have been displaced from their normal position in the cell by the vacuole, *a*, which has been drawn in from the ends of the cell and which is clearly separated from the cytoplasm by a membrane. A number of the granules have stained and have flowed together to form a large globule, *g*.

Fig. 4. The same cell as in figure 3. The neutral red globule, *g*, is very much enlarged and has become appressed to the vacuole, *a*. Stain is diffusing into the vacuole

Fig. 5. Same cell as figures 3 and 4. Much stain has diffused into the vacuole, giving the cell a decided red color. Although the neutral red globule, *g*, and the vacuole *a*, are distinguishable, the membrane separating them is much less distinct.

Fig. 6. A large neutral red globule has formed in the center of the cell. A number of smaller globules, *g*, may also be observed. One of these toward the bottom of the cell is slowly being absorbed by the large globule. The chloroplasts have bunched together in an amorphous mass below the region of the globules.

Fig. 7. The large neutral red globule of figure 6 has completely absorbed the smaller globules so that the entire cell is red.

Fig. 8. Protonemal cell some time after staining with neutral red. The chloroplasts have lost their typical shape and distribution. The vacuole, *a*, is quite removed from the ends of the cell and is sharply delimited from the cytoplasm by a membrane.

Fig. 9. The membrane separating the vacuole and globule has broken. The neutral red mass, *ag*, formed by this phenomenon is slowly pushing its way into the mass of chloroplasts and cytoplasm, *p*.

Fig. 10. The neutral red mass, *ag*, has advanced further into the cytoplasm, *p*.

Fig. 11. The neutral red mass, *ag*, is still slowly pushing its way into the cytoplasm, *p*.

Fig. 12. A protonemal cell after neutral red staining, showing several neutral red globules.

Fig. 13. Protonemal cell after neutral red staining, showing the manner in which the vacuole changes shape and how the normal distribution of chloroplasts and cytoplasm is disturbed.

SOME DEVELOPMENTAL CHARACTERS OF SPECIES OF
· LYCOPERDACEAE [1]

Delbert Swartz

INTRODUCTION

Most of the papers on the Gasteromycetes treat them from the standpoint of taxonomy; only a few deal with their morphology and physiology. Of these a few are limited to the Lycoperdaceae. The first reported study of Gasteromycetes was that of Corda (1842), who simply described the germination of spores of *Sphaerobolus stellatus* Tode. under natural conditions. Sachs (1855) was unable to germinate spores of *Crucibulum vulgare* Tul. Hoffman (1859) described the germination of the spores of the following species: *Lycoperdon echinatum* Pers., *Lycoperdon gemmatum* Batsch., *Bovista plumbea* Pers., and *Cyathus striatus* Willd. His later papers (1860a, 1860b) reported his failure to duplicate his first experiments. De Bary in 1863, although unable to germinate spores of *Phallus impudicus* L., gave us the first account of the development of *Lycoperdon sp., Bovista plumbea,* and *Geaster hygrometricus* Pers. (see De Bary, 1887). Pitra (1870) experimentally verified the previous observations of Corda on *Sphaerobolus stellatus*. Eidam (1875, 1876) produced an oidium-forming mycelium from spores of *Crucibulum vulgare* and *Cyathus striatus*. Hesse (1876) obtained a freely branching, oidium-producing mycelium from spores of *Cyathus striatus* which germinated in water after 18–24 hours. Brefeld (1877) grew mycelium of unnamed species of *Crucibulum* and *Cyathus* from single spores until fruit bodies were formed; these fruit bodies apparently developed in the usual way, but remained empty and failed to mature. Rehsteiner (1892) gave us the first critical study of species development, using *Lycoperdon gemmatum* Batsch., *Lycoperdon laxum* Bon., *Bovista plumbea* Pers., and *Geaster fornicatus* (Huds.) Fr. Rabinowitsch (1894) made a detailed study of *Lycoperdon depressum* Bon. Ferguson (1902a, 1902b) reported that she was unable to germinate spores of Gasteromycetes. Duggar (1901, 1905) grew tissue cultures of several species, but did not report the formation of any fruit bodies. Molliard (1909), using an entire peridiole of *Crucibulum vulgare,* eventually obtained the normal fruiting stage. He did not study the details of development. Cool (1912) reported her inability to germinate spores of Gasteromycetes. Cunningham published the details

of the development of *Lycoperdon depressum* (1926) and the development of *Geaster velutinus* Morg. (1927). Lohwag (1926) discussed the phylogeny of Basidiomycetes in some detail and based his conclusions on the Lycoperdaceae upon the work of Rehsteiner. The writer published the details of spore germination in *Lycoperdon pyriforme* (Schaeff.) Pers. (Swartz, 1928). Gaümann and Dodge (1928) consider the whole matter of the classification of the Gasteromycetes as unsettled and subject to revision when our knowledge of it is increased.

The writer hopes that the following account of the morphological development of five hitherto unstudied species will contribute something of value to the yet unsolved problems of the Lycoperdaceae.

MATERIAL AND METHODS

The following species were selected for study: *Calvatia saccata* (Fr.) Lloyd, *Bovista plumbea* Pers., *Lycoperdon Wrightii* Berk., *Lycoperdon pulcherrimum* B. and C., and *Lycoperdon pyriforme* (Schaeff.) Pers.

Material representing all stages of development of the fruit bodies was collected, killed in Flemming's strong solution, and imbedded in paraffin in the usual way. Better penetration by the killing solution was secured if the air was exhausted from the fruit bodies by means of a suction pump. The exhaustion of air made the fixation of fairly large fruit bodies possible and also was advantageous in preventing injured tissues. However, many of the larger fruit bodies were cut longitudinally into two parts before fixing.

Sections were cut $5\,\mu$, $7\,\mu$ and $10\,\mu$ in thickness, but it was soon found that the sections $7\,\mu$ thick were the most satisfactory. All the sections were stained with Heidenhain's iron-alum haematoxylin.

OBSERVATIONS

Calvatia saccata

Description of young fruit body. Young fruit body at first equal, becoming depressed globose above, plicate below and abruptly contracted into a long stem-like base; the base thick and stout, often lacunose, nearly equal and tapering downward. Cortex a thin coat of small, persistent, meal-like granules. Attached to ground by thin white rhizomorphs.

Mature fruit body. Maximum size 12–15 cm. high, 6–8 cm. in greatest diameter. Upper globose fertile region supported by a rather stout, sterile, stem-like base 4–5 cm. in diameter, noticeably constricted below fertile portion. Outer surface roughened due to the drying of the terminal cells of the peridial hyphae, dingy yellow with, at times, a purplish tint. More or less regular depressions on sterile base,—abundant above and less frequent below. Basal part comprising greater part of mature fruit body. In section, base is composed of numerous rather closely formed cavities which become closer where base and rhizomorph are joined. Fruit body attached to

substratum by rhizomorphs at maturity. Spore mass brown, somewhat darker than the sterile tissue of the base. Opening by rupturing of peridium, which falls away, exposing spore mass; rarely a pore formed at apex. Large sterile stem remains attached to substratum long after spores have been scattered.

Rhizomorphs. The rhizomorphs are short, thin, white threads which taper greatly as they spread out from the fruit body. They are much thinner and branch less frequently than those of *Lycoperdon pyriforme* as they penetrate the soil and other débris present on the forest floor.

In longitudinal section (fig. 1, pl. 29) there are three layers: the two outer ones which are very distinct, and the third inner one, composed of hyphae which are interwoven with the hyphae of the layer surrounding it. The outermost layer or cortex is clearly defined, and is composed of thick, blunt, deeply staining threads which are interwoven considerably at the small end of the rhizomorph; here it makes up the greater part of the total diameter. The internal hyphae at the tip of the rhizomorph are compact, and many of them do not extend to the extreme tip; others which do are lost in the mass of cortex hyphae which covers the tip. These cortex hyphae extend directly into a mass of loosely connected, undifferentiated mycelium mixed with soil particles and other débris. The origin of these thick, heavy hyphae of the cortex is shown in longitudinal sections (fig. 1). They are not differentiated internally, and are so brittle that it is doubtful whether or not they are alive. Deposits of material, unevenly laid down within these threads, give them a darker color and a wavy outline. They can be traced to the threads of the subcortical layer, and are sometimes connected to living hyphae.

The subcortical layer, located just beneath the cortex, is composed of very closely interwoven hyphae which are, for the most part, parallel to the longitudinal axis of the rhizomorph. They have thin walls and do not stain deeply; this fact indicates a less active or sparse protoplasmic content. The hyphae of this layer are continuous with those of the central core.

The central core of the rhizomorph is composed of hyphae which, in part, originate in the subcortical layer. As the thinner hyphae of this outer layer pass into the center, they increase greatly in diameter. Their protoplasmic content is richer and they stain more deeply. The hyphae walls are also thicker. The nuclei, which are too small to be seen in detail, stain more deeply; several of these tiny nuclei occur in each cell. Septa occur occasionally and large vacuoles are present in the cells. Here and there in these hyphae are deposits of materials which may be in the form of large, angular, deeply staining crystals. The origin of this material is not clear, but the crystals are similar in structure to bodies reported by Conard (1915) in *Secotium agaricoides* (Czernaiev) Hollos. A few thinner hyphae may enter from the subcortical layer, grow for a short distance in the central region and then turn back into the region from which they arose. Such hyphae do

not stain deeply. Because these hyphae of the rhizomorphs of this species branch and interweave a great deal, the central region is not as compact as it is in other species.

Formation of the fruit body. It is extremely difficult to collect material to show the actual initiation of a fruit body. Such difficulty is due to the advanced stages of the fruit body when proper identification is possible. It is necessary to study the attached mycelium to find the initials, and very often after some of the fruit bodies have matured such young stages can be found no longer. However, in one instance material showing the initial stages was collected.

The first indication of a young fruit body is an enlargement on a rhizomorph (fig. 2). As this enlargement increases in size it becomes nodular. This nodular structure is directly connected to, and covered with the hyphae of the cortex of the rhizomorph. The heavy, thick, outer cortex hyphae are very conspicuous at this time, and are much more numerous where the now forming structure is connected to the rhizomorph. The interior hyphae of this initiated carpophore are much interwoven, and are simply continuations of the hyphae of the interior of the rhizomorph. 'They are thin-walled and rather frequently septate; the entire structure is homogeneous throughout (fig. 3). The young initial gradually becomes larger due to an increase in the number of hyphae in the interior. After a relatively short time, specialized hyphae appear which grow upward and branch outward toward the periphery. The peridium is initiated at the apex from these hyphae (fig. 4).

. *Peridium.* The peridium is first distinguishable because of its reaction to stains rather than because of any noticeable morphological difference. The branching of the hyphae at first produces a loose arrangement of radial hyphae. The terminal cells of these hyphae which initiate the peridial layer soon become loosely connected and very irregular in shape. During its development the peridium is always more strongly developed in the apical region than toward the base. A second layer now develops just beneath the newly formed peridium. This layer is composed of loosely interwoven hyphae which are similar to the hyphae of the outer layer except they are not radial and do not stain as deeply. This layer does not differentiate further; instead it seems to serve as a base from which the outer hyphae of the peridium arise. As the fruit body expands the peridial hyphae become pseudoparenchymatous and this results in a much thicker layer.

The sterile base now elongates rapidly. At the same time the diameter of the fruit body increases considerably, more in the fertile than in the sterile portion. This increase results in a marked constriction at the connection of the fruit body and the rhizomorph. During the time of this expansion the sterile portion of the fruit body is covered by a layer of hyphae which are arranged quite differently than are the outer hyphae of the fertile part. These hyphae, although somewhat interwoven are chiefly parallel to the long axis. The layer thus formed is thin and never becomes pseudo-

parenchymatous. As the stem expands and elongates these hyphae bend outward, and a radial arrangement eventually results. This is not as clear cut as it is in the fertile part. Continued growth results in an extension of this radial arrangement to the basalmost part. The outward direction of the hyphae results in the formation of a collar around the fruit body at the junction of the fertile and sterile parts (fig. 5). The hyphae of the peridium dovetail with the outer hyphae of the sterile base in this region. Before the hyphae of the base turn outward, they grow upward for some distance; this upward extension prevents the radial arrangement of the component hyphae which is characteristic of the peridium proper. In the later stages of the formation of the sterile stem a deeply staining layer, situated just within the outer layer, is differentiated. The function of this layer is not clear, but because of the interwoven condition of the component hyphae it may perhaps function in contributing to the rigidity of the base. This layer, in some respects, is similar to the endoperidium formed by some of the lycoperdons.

At maturity, the peridium breaks up into irregularly torn pieces and falls away, thus exposing the powdery dry spore mass. When environmental conditions are unfavorable an apical opening, resembling the " pore " of the lycoperdons, may result. This is, of course, the exception, and the openings usually occur on the rounded surface rather than apically. Ordinarily the first ruptures of the peridium occur at the junction of the sterile and fertile portions.

In the above discussion the term peridium has been used to designate the structure covering the fertile globose portion. *There is no separation of the peridium into an endoperidium and an exoperidium,* as has usually been described. It seems advisable, however, to retain the term peridium rather than to present a new name for this covering. The presence of a one-layered peridium in this genus is significant and will be discussed later in the paper.

Gleba. In the earliest stages of fruit body development all of the tissue enclosed by the peridium is the gleba. In the initials of the fruit body, however, it cannot be distinguished as a morphological structure. The hyphae of the central part of the initial are intricately interwoven, thin-walled, many-septate and form a homogeneous mass. As increase in size takes place, the fertile portion becomes globose, and the interior hyphae become loosely interwoven.

Cavities soon appear in this rapidly enlarging body. They are irregular in shape and appear to be caused by the growth of the fruit body rather than by the dissolving of hyphae. They are formed first in the upper central region (fig. 6) and become larger; smaller ones appear toward the base. These cavities rarely have regular outlines at this stage because of the unequal proliferation of the hyphae. In this species the cavities are bounded by parallel hyphae which are more regularly arranged than those

in the lycoperdons. These parallel hyphae are most conspicuous when the cavities are being formed (fig. 7) and are the tramal hyphae which originate in the base of the fruit body, and wind about as they grow toward the apex. After the cavities become somewhat larger a rapid proliferation of hyphal branches takes place. These branches extend in all directions, and grow toward the outer limits of the cavities. Finally the ends of these branches form a regular palisade around the outer edge of the cavity. These hyphae again branch several times, and a number of hymenial units may result from one tramal hypha. This branching gives rise to a somewhat loosely interwoven zone between the trama and the hymenium. Rehsteiner (1892) has called this the subhymenial layer.

The cavities are not completely lined with basidia at one time; instead there is an uneven foundation of hymenium, and basidia are scattered around the edge of the cavities. Additional basidia are formed a little later and complete the hymenial layer (fig. 8). Tramal hyphae may give rise to the hymenium of several cavities as they wind from the base to the apex.

Occasionally stout hyphae, resembling those of the trama, pass from the hymenium on one side of a cavity, and become lost in the hymenium on the opposite side. Ordinarily such hyphae, quite common in this species, are not in the proper plane to be shown in one section of the fruit body. This may partly explain the opinions of other workers that cavity formation results entirely from the disintegration of hyphae rather than by unequal proliferation and separation of the glebal hyphae.

The cavities may become completely lined with hymenium before they show much increase in size. Increase in size takes place in two ways: (1) by the intercalary formation of new basidia and (2) by the coalescence of neighboring cavities. This coalescence occurs frequently. Two cavities, each having a continuous hymenium, are pushed closer and closer by the insertion of new basidia. Soon the tissue separating them begins to disintegrate and eventually completely disappears. For a short time the continuity of the hymenium is interrupted, but branches of the tramal hyphae soon give rise to additional basidia. This new hymenial layer is similar in appearance to the layer which had been formed earlier. There is no way to determine whether or not these new basidia form spores simultaneously with the old. New cavities may form between old ones, and become lined with basidia in the usual way.

Toward the base of the gleba undifferentiated tissue may still be seen. Cavities may be formed in this tissue even after those in the upper regions are completely mature. These are, however, ordinarily smaller than the first formed ones.

When the cavities have reached their maximum size, spores are produced —four on each basidium. At first they are small spherical swellings on the ends of the sterigmata; later the exosporium becomes thicker and roughened. The roughening is most conspicuous after the spores are mature. The

nuclei are too small to be seen satisfactorily during the process of spore formation.

Spore formation uses up a great deal of the trama and the subhymenial layer. A disintegration of these tissues now takes place. Many capillitium threads are formed. The moisture content is much reduced, and as a result the spore mass becomes dry and powdery. The peridium becomes soft and leathery, and spore dispersal takes place as described above.

Capillitium. The threads of capillitium arise from the growing tramal hyphae (fig. 9, pl. 30). From one of these hyphae a cell is cut off by a septum; the protoplasmic content of the resulting cell is greatly reduced, and crystalline material is deposited on the outside, making the outline irregular. At the same time granules appear on the inside. The deposits cause an increase in size and the structure becomes dark in color. Such threads occur in the peridium and in the sterile base. The formation of capillitium is not by the method suggested for *Lycoperdon depressum* by Cunningham (1926). Scattered threads of capillitium appear during the differentiation of the fruit body, but due to the disintegration and disappearance of the trama and subhymenial layer, they become more conspicuous. Capillitium evidently serves in the disposal of waste material rather than in spore dispersal as has been suggested.

Sterile base. Fruit body initials have no sterile base. A dense compact zone of hyphae which is connected to the interior of the rhizomorph gives rise to the base. The hyphae of this zone elongate considerably and curve outward. The interior of the resulting structure possesses cavities which are in every way comparable to those which develop in the fertile portion. Sometimes these cavities elongate laterally in the zone of transition between the fertile and sterile portions. Such cavity elongation does not occur in all fruit bodies, and it is only upon drying that the zone of separation becomes evident; it cannot be located in growing fruit bodies.

Threads of capillitium appear soon after the cavities are lined with hymenium. They first appear in the tissue between the cavities immediately after a period of great nuclear activity in the sterile hyphae which line the cavities. Since there is no disappearance of any tissue in the base, these threads remain scattered and are not particularly conspicuous.

Bovista plumbea

It is commonly known that fruit bodies of *Bovista plumbea* rarely occur in sufficient numbers, or in proper condition for a developmental series (Lloyd, 1902). In October, 1927, while passing through a pastured pine plantation east of Ann Arbor, the writer was fortunate enough to find representative fruit bodies of all ages growing under and among the fallen pine needles. The larger, more mature specimens were plainly visible as they emerged from the débris, but the smaller and younger ones were completely covered.

Description of fruit bodies. Young fruit body at first spherical, or depressed spherical, white; slightly areolate. Covered with a thin white cortex which early breaks up and falls away. Sterile base absent; fruit body weakly attached basally to substratum; easily separable.

Mature fruit body. Surface at first smooth, gradually becoming areolate with exoperidium easily separable and rupturing at boundaries of these areolae when drying occurs; and falling away in irregular pieces, thus exposing the soft, leathery, slate-colored exoperidium which persists as the tough papyraceous covering of the fruit body. Spore mass comprises the greater part of the plant when dry; microscopically composed of pedicellate spores mixed with capillitium. Opening, apical; sterile base, none. Fruit body much constricted at base, forming a thickened layer intermixed with soil particles, etc. Entire fruit body easily separable from substratum because of weak rhizomorphic connection.

Rhizomorph. Because of the ease with which fruit bodies may be separated from the substratum, it is very difficult to collect specimens showing a fruit body attached to a rhizomorph. The connection is so weak that occasionally fruit bodies become detached while they´ are still white and undifferentiated. When this happens they mature rapidly in the usual manner. There are two well developed layers of the fragile, slender, white rhizomorph. These layers are a central core, and an outer layer surrounding it; none of the blunt, dark cortex hyphae similar to those of *Calvatia saccata* are present. The sections studied were made of a rhizomorph still attached to a well developed fruit body.

Formation of the fruit body. The fundament of the youngest fruit bodies consists of an irregularly spherical, homogeneous mass of fairly large, much interwoven hyphae. Soil particles, old spores and other foreign matter cling to this mass of hyphae, and make cutting so difficult that badly torn sections often result.

Exoperidium. The initial of the fruit body described above soon becomes covered with a tangentially parallel layer of hyphae. The hyphae just beneath this covering branch profusely to form a zone of radial hyphae which are continuous with the hyphae of the outer layer. Accordingly the exoperidium is now composed of two different layers (fig. 11). The inner zone now becomes thicker and distinctly pseudoparenchymatous. The hyphae of the interior of the fruit body grow and cause the interior to expand, and the outer hyphae of this interior region enter into the formation of additional pseudoparenchyma in the inner zone of the exoperidium. The outermost layer gradually sloughs off with this increase in size, and is replaced by additional hyphae from the pseudoparenchyma. Sections show the two outermost layers to be easily separable from each other. At length the exoperidium is torn into ragged pieces which fall off. Ordinarily this starts at the apex, and gradually progresses toward the base.

Endoperidium. A continuous narrow zone of deeply staining hyphae

appears at the inner limits of the exoperidium. High magnification shows these hyphae to be loosely interwoven, and of greater diameter than those of the gleba. This zone becomes thickened, and the hyphae more compact due to the growth of new hyphae and the outward pressure of the developing gleba. Some of the hyphae on the outer edge are continuous with the hyphae of the exoperidium; while those toward the interior enter the gleba. However, this connection between the two peridia soon becomes weakened, and in my sections this weakened zone is demonstrated by the frequency with which the two peridia are separated. Very few sections could be made which were not separated at some place or other. When the endoperidium is well developed the hyphae composing it become darker, although the branches on either side may remain hyaline. The hyphae at the apex pull apart, and an opening for the escape of spores results.

Gleba. The differentiation of the gleba ordinarily begins very early. In this respect *Bovista plumbea* corresponds very closely to *Bovista nigrescens* Pers. (Rehsteiner, 1892). This differentiation is directly dependent upon environmental conditions and not upon the size of the fruit body. In this species as in *Calvatia saccata* glebal differentiation is more easily disturbed by unfavorable conditions than is the formation of the peridia.

Glebal differentiation is initiated in this species in much the same way as Rehsteiner (1892) found in *Bovista nigrescens*, and is similar to that of *Calvatia saccata.* A few points of difference should be pointed out. The hyphae of *Bovista plumbea* are generally coarser than similar hyphae in the species mentioned above; this is especially true of the tramal hyphae. There is no particular place for the formation of the first cavities, but they are usually situated in the central region with fewer toward the base. They appear promiscuously and are soon lined with hymenium; this is due to the branching of tramal hyphae. The formation of the hymenium takes place in a somewhat different manner than in any other species. The lateral or terminal branching of the tramal hyphae forms an umbel of secondary branches which have somewhat rounded ends. These grow toward the cavities and give rise to the basidia which line the cavity. Such groups of branches occur quite often, and as a result the subhymenial layer is thinner than in the other species.

As the fruit body increases in size new cavities appear as described for *Calvatia saccata,* and eventually the entire glebal region is a complicated network of cavities of various sizes. Such cavities coalesce quite commonly. The hymenium is similar throughout. No mature spores were observed until differentiation was almost complete. Rehsteiner (1892) saw mature spores very early in young fruit bodies of *Bovista nigrescens.* The spores in my studies were at first colorless, spherical and borne on short sterigmata. Later these sterigmata elongate, and remain attached to the spores when they are freed; the pedicel, therefore, is the sterigma which remains attached to the spore. Sometimes before detachment, sometimes after, the spore wall is thickened, and assumes a golden-brown color.

Capillitium. During later stages of spore formation the threads of capillitium become more conspicuous. They apparently are of hyphal origin much the same as *Calvatia saccata,* but the definite manner of their formation could not be determined.

Sterile base. The genus *Bovista* has always been separated from *Lycoperdon,* in part, by the absence of a sterile base. In the sections of *Bovista plumbea* examined in these studies a reduced sterile base was found, although it did not have the appearance of the sterile base of the lycoperdons, and cannot be rightly so called. In specimens not completely mature, the endoperidium and the exoperidium at the base are quite different from the peridia on the remainder of the fruit body. In some cases the sterile basal region was more manifest than in others. Where it occurs it is composed of closely interwoven vertically arranged hyphae. Here and there certain hyphae bend outward to the periphery, where they grow into the peridia. This mat of interwoven hyphae gives rise to a differentiated region in which there are no cavities, thus showing another difference from closely related genera. From all evidences this region of sterile tissue cannot be considered a true sterile base.

Dehiscence. The apical hyphae of the endoperidium become loosely arranged and an irregularly torn opening results. As the fruit body rolls from place to place the spores are freed. It is not uncommon to see old fruit bodies still containing spores one year after they have been formed.

Lycoperdon pulcherrimum

Description of young fruit body. Young fruit body at first subglobose to obovate. Covered with a cortex of long white convergent spines 3–4 mm. long; the spines connivent, becoming darker in age. Attached to substratum by well developed sterile base without deep rooting structure.

Mature fruit body. Fruit body obovate and covered with a well developed cortex of convergent spines except at the base. Spines becoming dark, somewhat curved, and falling away at maturity, exposing the smooth, silky endoperidium. Spore mass at first white, becoming brown in age. In median longitudinal section a marked constriction from the globose fertile region to the sterile base is visible.

The extreme shagginess of the spines of this species is approached by two species—*Lycoperdon echinatum* and *Bovistella pedicellata.*

Rhizomorph. The rhizomorphs are not strongly developed, and break away from the fruit body very easily at the time of collection. When they do remain attached they are often broken off, and lost during the process of killing and fixing. Such circumstances made it necessary to base the following remarks on examination of unstained material. The rhizomorphs are at first white, much branched and they extend into the soil some distance from the fruit body. Under the microscope two layers are visible, one a rather loosely connected layer of cortex hyphae having the same general

characteristics as those described for *Calvatia saccata;* the other layer is composed of very thin hyphae which run parallel to the longitudinal axis. Neither layer is strongly developed.

Development of fruit body. Fruit bodies of this species arise in much the same way as in *Calvatia saccata;* either laterally or terminally from a rhizomorph or a branch of it. Sections through the youngest available fruit bodies show a compact, homogeneous mass of closely interwoven hyphae. The peripheral hyphae become radially arranged very early and form the initial of the exoperidium.

Exoperidium. Radial hyphae appear simultaneously over the periphery of the fruit body. This differs somewhat from the condition in *Lycoperdon pyriforme* and *Calvatia saccata,* where no peridium is formed around the sterile base. These hyphae, at first, have a uniform diameter, and their tips form a compact, slightly interwoven layer which completely encloses the fruit body. The walls of the hyphae are thin, and the protoplasmic content of the cells seems somewhat reduced. As in *Bovista plumbea,* the inner part of the layer becomes pseudoparenchymatous after the endoperidium is formed.

Gradually the interior of the fruit body expands, and some of these interior hyphae proliferate into the developing exoperidium and assume the characteristic radial arrangement. Due to this internal expansion the outermost hyphae of the exoperidium separate, and fissures appear between groups of them; this gives rise to conical groups of cells which later become irregularly shaped because of their loose connection. With additional expansion the fissures become deeper and extend into the pseudoparenchyma. The exoperidium is naturally very thick, and the fissures are correspondingly deep. The conical arrangement of hyphal cells between the fissures is really due to the spines which are so characteristic of this species. It follows then that the longest spines simply result from the deepest fissuring.

After these long spines have formed, the entire outer layer is very easily disturbed during cutting, and tends to separate from the endoperidium. The cells composing the tips of the cones are not as easily separable from each other as they are in *Lycoperdon pyriforme;* this indicates a closer relationship of these cells, due, at least in part, to the somewhat interwoven condition of the hyphae from which they are formed. This partially explains the curved tips and convergence of the spines. After the endoperidium is formed the fissures keep pushing in until they reach it. The endoperidium is, however, never split. In later stages the cortex of spines falls off and exposes the endoperidium.

Endoperidium. Shortly after the differentiation of the distinct outer layer which gives rise to the exoperidium, a zone of thin, loosely interwoven hyphae appears some distance beneath it. This zone completely encircles the interior; the component hyphae do not stain deeply and have a thin wall. The branching of the original hyphae of this layer, as well as the entrance of new hyphae from either side, causes it to increase in thickness. The

hyphae on the outside of this layer are continuous with the hyphae of the exoperidium; and those on the inside are continuous with the yet undifferentiated gleba. Just within this newly formed endoperidium there is a growth zone which remains unchanged until after the gleba has differentiated.

Gleba. The gleba is formed as in *Lycoperdon pyriforme* and *Calvatia saccata.* Cavity formation, however, begins much earlier, and the first ones appear about the same time the endoperidium is laid down. They appear between rapidly growing hyphae and at first are irregular in outline. They are formed promiscuously throughout the upper central part of the gleba, and continue to form until the greater part of the gleba is used up. Their increase in size takes place as in *Calvatia saccata* and *Bovista plumbea.* The hymenium which eventually lines the cavities arises from a very poorly developed trama, whose component hyphae show only a very slight tendency toward a parallel arrangement. Because of this the subhymenial layer is weakly developed; the hyphae of this layer resemble those of the trama. There is no noticeable transition from one tissue to the other. Here and there, as in *Calvatia saccata* and *Bovista plumbea,* large, thick-walled hyphae pass through the cavities from one hymenium to the other.

The trama ordinarily disintegrates during the formation of spores, and mature spores may be seen in the cavities before differentiation is complete. This corresponds to *Bovista nigrescens* as described by Rehsteiner (1892).

Capillitium. Capillitium is formed in this species exactly as described for *Calvatia saccata.*

Sterile base. The sterile base was poorly developed in all the fruit bodies studied. There is no definite line of demarcation marking the transition from fertile to sterile tissue. Cavities are formed in the sterile base, but are not lined with a palisade of sterile hyphae as in *Calvatia saccata.* The hyphae making up the base are thin and much interwoven; they originate in the central hyphae of the rhizomorph, pass upward through the sterile base into the fertile region, and there they become interwoven with the hyphae of the peridia.

The outer covering of the sterile base is a layer of radial hyphae. This covering layer is not as thick here as around the fertile portion (fig. 12). The splitting of this layer, therefore, does not give rise to fissures as deep as those near the apex, and as a result a thick cortex of spines is not produced.

Dehiscence. When the fruit body has matured the apical hyphae of the endoperidium become rearranged and are pulled apart. This gives rise to the apical pore through which the spores escape.

Lycoperdon Wrightii

Description of young fruit body. Fruit body spherical, rarely oval; irregularly compressed due to close association with neighboring fruit bodies; ordinarily sessile, rarely with a short stalk. Cortex very rough, spiny; spines

thickly clustered, bending together at apex; becoming dark very early, and imparting a dull yellowish color to the young fruit body.

Mature fruit body. Mature fruit body covered with a thick spiny exoperidium; exoperidium breaking into rough, ragged, unevenly torn pieces which are readily separable from the endoperidium when dry. Endoperidium papyraceous, very tough, persistent, at first orange colored. Spore mass olive in color and occupying the greater part of the fruit body. Exteriorly no definite zone of transition from the fertile to sterile portion. Sterile base present, very poorly developed, and composed of large, unevenly formed cavities; persistent, often remaining attached after spores have been scattered.

Rhizomorph. It was necessary to examine free hand sections of the rhizomorphs, as they are so delicate and easily separable from the fruit bodies that they were invariably lost during dehydration and infiltration. They are thin, branched, and covered with outgrowths of short white hyphae. In all other respects they correspond closely to the rhizomorphs of *Lycoperdon pulcherrimum.* The cortex of dark-colored, blunt hyphae is not as strongly developed in this species as in *Lycoperdon pyriforme* and *Calvatia saccata.*

Development of fruit body. Adhering soil particles make it extremely difficult to cut satisfactory sections of fruit body initials. A few sections were obtained which were not badly torn, and which showed the structure of this mycelial mass very well. It is loosely interwoven and composed of fairly large hyphae throughout. In the youngest fruit bodies, the outermost hyphae are arranged radially and extend around the fruit body as far as the base. The hyphae at the base are more compact, and arise directly from the rhizomorph. These hyphae grow upward and bend toward the periphery.

Exoperidium. The radial hyphae mentioned above form the first exoperidial layer. These hyphae are coarse, and have a thin wall. They branch frequently and soon become somewhat interwoven. As development continues the exoperidium becomes evenly thickened except at the base. During this thickening the hyphae become more interwoven, and additional hyphae enter from the undifferentiated interior. At this stage the exoperidium composes the greater part of the total diameter (fig. 13). Later the exoperidium becomes pseudoparenchymatous. The gleba now differentiates rapidly, and since the outer layer of the exoperidium does not keep pace with this internal expansion, fissures appear, and as a result spines are formed. This process is similar to that in *Lycoperdon pulcherrimum.* In cross section the fruit body resembles *Bovista plumbea* except that the latter does not have spines, but instead the outermost hyphae are arranged tangentially parallel. There are places in the exoperidium of *Lycoperdon Wrightii* where the hyphae are loosely arranged (fig. 14); the areas indicate the cracks which are formed later before the exoperidium falls off. Areas like these are not formed in *Bovista plumbea;* otherwise the two exoperidia are somewhat similar. After the exoperidium falls off at maturity, the endoperidium remains as the covering for the spore mass.

Endoperidium. The formation of the two peridia is simultaneous. The endoperidium, at first, is simply composed of a few thin-walled hyphae which are nearly parallel, and are tangential. Gradually it becomes more interwoven, compact and thickened. In this case, as in the others previously described, the endoperidial hyphae are continuous with the exoperidial hyphae on the outside and with the glebal hyphae toward the interior. This layer of hyphae extends completely around the fruit body, and forms a *definite line of separation between the globose fertile region and the more constricted sterile base.* At maturity an apical opening is formed.

Gleba. The formation of the gleba is similar to that of *Calvatia saccata.* A complicated network of cavities results; these become lined with hymenium which arises from a poorly developed trama. This trama is similar to that of *Lycoperdon pulcherrimum.* Spore formation and subsequent differentiation takes place as in the preceding species.

Sterile base. An unusually small part of the tissue in this species is sterile, and this is confined to the base. The hyphae of the basal zone show no change until after the other tissues are almost completely formed. Cavities are present here as in other species with sterile bases, but they are correspondingly fewer in number. As has already been pointed out, the sterile base is separated from the fertile part by the continuous endoperidium. The hyphae of this region appear white in mass. As in the preceding species the base remains attached to the ground after the spores have been scattered.

Dehiscence. At maturity an apical opening is formed as in the preceding species. The spores are freed through this apical opening.

Lycoperdon pyriforme

Description of young fruit body. Fruit body pear-shaped from the beginning; covered with a cortex of minute, fasciculate, subpersistent spines evenly scattered over the globose fertile portion. Sterile base fairly well developed in the younger stages; attached to woody substratum by a long, white, fibrous mycelial strand.

Mature fruit body. Fruit body pear-shaped with a fertile globose head supported by a fairly well-developed sterile base; partially covered with dirty whitish spines. Pore formed apically. Gleba changing from white to yellowish brown at maturity. At first there are two distinct peridia, but only one ordinarily is persistent until fruit body is fully mature. No definite line of separation between the brown spore mass and the sterile base. Sterile base attached to a well developed rhizomorph. Rhizomorph ordinarily on wood or in ground rich in decaying wood.

Rhizomorph. The rhizomorphic strands, which are more fully developed in this species than in any other, are made up of tough cords of white mycelium. They commonly grow on wood or in soil rich in decaying wood. A relatively large section of the rhizomorph ordinarily remains attached to the fruit body. In longitudinal section the rhizomorph has three layers.

45

The outermost layer is the cortex, which is composed of large, blunt, unbranched, dark-colored, loosely interwoven hyphae which make up one-fourth to one-third of the total diameter. These hyphae have no cross walls and are devoid of protoplasm. They arise from the hyphae of the layer just beneath, and are somewhat interwoven with them where the layers unite. The uneven thickening of their walls gives them a wavy appearance. These cortex hyphae have a protective function, and are fairly easily disturbed during handling. A much thicker layer of these hyphae covers the tip of the rhizomorph.

The subcortical layer is composed of thin-walled, sparsely septate, and rarely branching hyphae which run parallel to the longitudinal axis. They are uniform in shape and size, and are but little interwoven. They also contain a reduced amount of protoplasm. Such compact, long-celled hyphae doubtlessly strengthen the rhizomorph.

The central core is composed of little branching, multinucleate hyphae which are very rich in protoplasm. These hyphae stain deeply and sometimes there is a continuity of protoplasm between adjacent cells of the same hypha. There are somewhat larger, deeply staining bodies of two shapes within the central cells. These bodies are either spherical or cubical and stain very deeply. Similar bodies were called protein crystals by Conard (1915) in rhizomorphs of *Secotium agaricoides.*

At the tips of the rhizomorphs the two inner layers converge, and stain more deeply, thus indicating a richer protoplasmic content. The tip is covered with a compact, thick layer as in *Calvatia saccata.*

Formation of fruit body. Fruit bodies arise either laterally from rhizomorphs or terminally from branches of them. Obtaining material to show the first initial stages is difficult and uncertain. The first evidence of the new fruit body is a modification of the hyphae of the rhizomorph. Such groups of modified hyphae become interwoven, expand and give rise to nodular swellings. Expansion and enlargement of these hyphae continue until the nodular structure protrudes slightly on the rhizomorph as in *Calvatia saccata* (fig. 2). A more rapid expansion of the interior of this structure now forces the exterior hyphae outward, and the increased internal pressure causes the cortex to become thinner, but the cortex hyphae are still continuous with the outer layer of hyphae of the rhizomorph. The young fruit body, which is at first visible only through a hand lens, now becomes visible to the naked eye. With the exception of the adhering cortical hyphae the young fruit body has remained homogeneous throughout; it is composed of intricately interwoven hyphae rich in protoplasm. Differentiation now takes place rapidly.

Exoperidium. The outermost hyphae at the apex of the initial become loosely arranged, and, as expansion continues, a layer of radially arranged, thin-walled hyphae gradually appears. At first this change is limited to a very narrow apical region. This layer becomes the exoperidium, which

eventually covers the entire fruit body, and it is always more strongly developed at the apex than elsewhere. For a relatively short time it remains intact, but the outermost cells gradually lose their protoplasm, become irregular in shape and very loosely connected. The surface growth ceases and the outer layer becomes fissured, giving rise to the conical warts attached to the endoperidium (fig. 15). These warts are made up of loosely connected cells which may fall off, and as a result the warts do not keep their regular conical shapes. The fissures penetrate radially, and eventually the layer of pseudoparenchyma which makes up the interior of the exoperidium is involved. New exoperidial hyphae form continually by the outward branching of the hyphae of the endoperidium. By this time the upper portion of the fruit body is covered by the developing exoperidium, which never completely extends to the base. The hyphae of the exoperidium become interwoven with the outermost hyphae of the base. This interweaving of the hyphae forms a rather conspicuous shoulder around the fruit body. Due to the splitting of the outer layer at the exoperidium, it gradually falls off until at last nothing remains of it except a layer of pseudoparenchyma. This layer also gradually becomes thinner at maturity.

Endoperidium. Shortly after the exoperidium is initiated at the apex, a second layer, recognizable by a deeper staining reaction, is formed. At first this layer is confined to a rather restricted zone at the apex, but it gradually extends downward toward the shoulder. The component hyphae are parallel at first, but due to rapid branching they soon become interwoven, and assume a tangential position. Some of these hyphae turn outward and become a part of the exoperidium; others turn inward and become a part of the gleba. This layer of hyphae just described is the endoperidium. It gradually becomes more compact because of the outward pressure exerted upon it by the developing gleba, and persists in this condition until the fruit body matures

During the later stages of development, after the sterile base has been delimited, a growth zone appears in the region where the peridial hyphae meet the outer hyphae of the sterile base. From this zone new endoperidial hyphae are formed. These newly formed hyphae soon become interwoven with the original endoperidium, and gradually become compact. This gives rise to the distinct endoperidium which surrounds the sterile base at maturity.

Gleba. The gleba is at first composed of a compact mass of intricately interwoven hyphae. These hyphae are rich in protoplasm, and are similar throughout. Rather early in the development of the fruit body the hyphae of the interior apical region become loosely interwoven, and two distinct types of hyphae appear. One type is very coarse and always originates in the basal region, bends outward and grows toward the periphery. In some cases these hyphae extend to the endoperidium. The other type of hyphae is smaller and more nearly resembles the hyphae of the gleba initial; they are multiseptate, freely branched and thin-walled. The larger hyphae are only occasionally septate and branch very rarely.

Cavities are first formed in this region near the apex, and later gradually appear toward the base. *These cavities are really interspaces between hyphae and arise by mechanical splitting.*

While the cavities are being formed a strongly developed network of bundles of parallel hyphae appears in the interior. These bundles are made up of four or five hyphae which by increase form the trama. These tramal hyphae branch, and the branches grow toward and into the periphery of the cavity. Here they form a palisade layer. The development of this layer is uniform in some places, but the periphery of the cavity is not lined with these cells at exactly the same time. The subhymenium lies between the hymenium and the trama, and is a tissue of loosely interwoven hyphae which are also branches of the trama.

While the hymenium is being formed a new layer of tissue is differentiated just within the endoperidium. It consists of much branched, interwoven hyphae. There are no cavities in this tissue at first, but later they appear.

New cavities with hymenium arise in many places between older ones. They sometimes fuse when two are close enough together. This fusion, or anastomosis as it has been called (Rehsteiner, 1892), results from the disintegration of the hyphae between. This is the same in all the species studied. After the greater part of the gleba has been used up, cavities begin to form in the secondary tissue just within the endoperidium. The manner of formation is somewhat different here. Basidia appear first on the side of the cavity toward the interior. This condition persists for some time, and is similar to that described for the early stages of cavity formation in *Hysterangium clathroides* as described by Rehsteiner (1892). Gradually, however, the hymenium spreads over the outer sides of such cavities.

Spore formation. The basidia show the effects of lateral pressure by flattened sides, but the ends are still rounded. A short time after the basidia appear, a septum forms at the base. The basidia are usually four-spored, and the first spores appear on the first-formed basidia. In some of the spores nuclei may be seen readily; in others they cannot be seen. Cunningham (1927) explains this situation by assuming that nuclei are present but that they are not stained.

After the spores are formed a rapid disintegration of all the remaining gleba hyphae and basidia takes place, leaving nothing except spores, capillitium and a few scattered crystals. This remaining mass is surrounded by the endoperidium which has become tough and paper-like.

Capillitium. Capillitium is formed in this species as in *Calvatia saccata.* It is very definitely of hyphal origin, and becomes conspicuous only after most of the gleba has been used up in spore formation.

Sterile base. The hyphae in the basal region remain comparatively inactive during the differentiation of the gleba, and have an upright position parallel to the long axis of the fruit body. An elongation accompanied by an increase in thickness takes place in this region. Prior to this development

the reduced exoperidium of the base has not been differentiated, and the enclosing hyphae are similar to the outer hyphae of the rhizomorph. A few cortex hyphae adhere to this layer, but as elongation continues an exo-peridium-like tissue is formed near the point of union of the fertile and sterile tissue. This becomes the outer covering of the mature sterile base, but it is much reduced when compared to the exoperidium of the fertile part. In addition it does not extend very far down along the side of the sterile base. In the interior of this region, cavities which become lined with basidia are formed. Such cavities here are longer and do not anastomose freely, and the basidia are more regular. In all cases, however, these cavities are not completely covered by basidia and they do not stain as deeply as do the spore-producing basidia of the fertile cavities. The layer from which these hyphae arise is ordinarily poorly developed. Toward the lowest part of the base the smallest and most poorly developed cavities are formed. At maturity such cavity formation has continued until they are present al-most to the region of attachment to the rhizomorph. These later-formed cavities are rarely lined with basidia. There is no definite line of demar-cation between the fertile and sterile parts of the fruit body; no morpho-logical explanation can be offered for the columella as it is seen in mature specimens.

Dehiscence. As the tissues of the gleba disintegrate, the endoperidium becomes modified at the apex. The hyphae become much looser and the remaining tissue becomes much thinner here than elsewhere. In this small area the hyphae are eventually pulled apart, and in this manner the pore is formed. Just why it should take place at this point is not clear.

DISCUSSION

Atkinson (1914a, 1914b, 1914c, 1916) in his studies of certain agarics pointed out the advisability of studying the development of closely related species of fungi in order to get a complete working knowledge of their relationships. His results showed that even though species bear a close resemblance externally, their methods of internal development are not always the same. The same fact holds true in our study of the Lycoperdaceae; similar species may develop quite differently.

Bonorden (1857) and Tulasne (1842) recognized that unless fruit bodies have had fairly favorable conditions for development, considerable difficulty may be encountered in placing them in their proper taxonomic positions. Rehsteiner (1892) and the writer (Swartz, 1929) have found that unfav-orable environmental influences affect the internal development much more than the external. It seems well, in view of these facts, to state that only healthy and normally developed fruit bodies should be used as the basis for any study whatsoever. For that reason the utmost care was used in selecting the material for this work.

Lloyd (1902), Rehsteiner (1892), and Coker and Couch (1928) have

stated that the peridium of species of Lycoperdaceae may be separated into two layers at least—namely, an inner layer, which is the endoperidium, and an outer layer, the exoperidium. In the genus *Geaster* three layers are ordinarily present. From the foregoing developmental studies, it is evident that the situation in *Calvatia saccata* is different. The peridium in this species is composed of only one layer; there is, in addition, relatively little sculpturing on the exterior. This layer is, without doubt, the endoperidium; and the sculpturings and markings occupy the position ordinarily taken by the exoperidium of other species. Such a modification seems logically of sufficient importance to substantiate a separation of the genus *Calvatia* from the genus *Lycoperdon*. In *Lycoperdon pulcherrimum*, where the youngest fruit bodies resemble *Calvatia saccata* quite closely, two very clearly defined layers develop. The exoperidium is especially well developed, and is covered by a cortex of long convergent spines. The differentiation of the exoperidium is followed later by the formation of the endoperidium. The endoperidium is never fissured, even after all of the tissue of the exoperidium becomes involved. The exoperidium of *Lycoperdon pyriforme* is, however, simpler than that of the foregoing species. It is similar for the most part, but is not so thick and has a looser arrangement of the cells making up the spines. The looser arrangement of the cells allows the spines to behave in like manner. In this species the pseudoparenchyma is not necessarily fissured; it sometimes persists until maturity as an outer covering for the endoperidium. The production of a cortex of spines in *Lycoperdon Wrightii* is similar to the preceding, but the peridium is quite different. The shedding of this outer layer corresponds more closely to the same process in *Bovista plumbea*, where the exoperidium reaches the highest development of any species studied. In *Bovista* areolae are formed which serve in the same capacity as the weakened areas in *Lycoperdon Wrightii*. The exoperidium of each species ruptures in these specialized areas. In addition to the rupturing in these areas, the two peridia often separate here, too. This separation occurs only after the formation of pseudoparenchyma, and it should be remembered that this tissue may be pulled apart more easily than any other tissue of the fruit body. It does not seem a very great step from this arrangement of peridial layers in *Bovista* to the three-layered peridium which Rehsteiner (1892) has described in *Geaster*. In *Geaster* the separability of the layers is quite evident; the layers do not necessarily fall away; but on the other hand, they persist and become reflexed. From the evidence at hand it appears that the exoperidium in *Calvatia saccata* has become so reduced that the adhering meal-like granules are the sole remains of it. No structures are formed in this species which can be considered homologous with the spines of *Lycoperdon pulcherrimum*, *Lycoperdon Wrightii*, or *Lycoperdon pyriforme*. These spines apparently should be considered homologous with the outer layer of tangentially arranged hyphae in *Bovista* and corresponding to the outermost layer in *Geaster*, where the outer layer reaches the maximum development in the Lycoperdaceae.

The endoperidium of *Calvatia saccata* is the outer rind, and it has been developed strongly enough to assume the function of the exoperidium. In *Lycoperdon pyriforme* the endoperidium is initiated at the apex and develops until it completely encompasses the fertile globose portion and becomes interwoven with the exterior hyphae of the sterile base. *Calvatia saccata* does not show this relationship to the same degree shown in every species of Lycoperdon studied. In every other species the hyphae of the endoperidium are continuous with the regions on either side; this connection may be rather loose because of the separability of the pseudoparenchyma. The compactly interwoven hyphae in the later stages of the development of this tissue explain the tough, leathery consistency of the mature endoperidium.

In *Bovista plumbea* and in *Lycoperdon Wrightii* the endoperidium is one of the first structures laid down, and it soon extends completely around the fruit body. This continuation of the endoperidium reminds one of the " grenz-linie " described for *Lycoperdon depressum* by Rabinowitsch (1894). This is not present in any of the other species studied.

The gleba, during differentiation and at maturity, is similar in all species studied. In every species the hymenium arises from a trama. In *Lycoperdon pyriforme* and *Calvatia saccata* the trama is well developed and consists of organized groups of branching, parallel hyphae. The trama is not so well developed in *Bovista plumbea* and it is very poorly formed in *Lycoperdon Wrightii* and *Lycoperdon pulcherrimum*. There is a definite layer of subhymenial hyphae in *Lycoperdon pyriforme* and *Calvatia saccata;* this layer is not well developed in *Bovista plumbea* and is inconspicuous in *Lycoperdon Wrightii* and *Lycoperdon pulcherrimum*. In the last two species it cannot be distinguished from the trama.

Cavity formation is similar in all species except *Bovista plumbea,* and the coalescence takes place similarly in all species. Rehsteiner (1892) pointed out that the basidia of the then investigated species of *Lycoperdon* and *Bovista* were similar. In our species this remains true. Spore formation is similar throughout the range of species. Rehsteiner also pointed out that glebal differentiation in *Geaster fornicatus* is similar to that of the lycoperdons and bovistas.

The formation of capillitium in our species of Lycoperdaceae is much different from capillitium formation in Myxomycetes as reported by Harper (1914) and Harper and Dodge (1914). The threads are often somewhat similar in appearance, but in the Lycoperdaceae they arise from growing hyphae. The workers mentioned above do not find this to be true in Myxomycetes. In all of our species capillitium formation is similar. No connection between capillitium and peridium was found in any species, as indicated by Rehsteiner (1892) and Cunningham (1926). Capillitium in the Lycoperdaceae ordinarily appears first in the trama. As the trama is used up in spore formation and subsequent disintegration, these threads become more conspicuous. Their irregular outline, as well as the thickening

of the wall and the occurrence of crystalline deposits with them, has led to the conclusion that they are more associated with the disposal of waste products than with the dispersal of spores. Rehsteiner (1892) found that similar deposits in related species were calcium oxalate. He found these crystals more or less regularly arranged along capillitium threads, particularly in *Bovista nigrescens*. The same is true for the species described in this paper. Cunningham (1927) stressed the appearance of capillitium threads in the region of the endoperidium. Such threads occur in similar locations in our species, but not in sufficient numbers to deserve special mention; instead, they occur scattered throughout the gleba. In *Bovista* the capillitium consists of star-like groups of hyphae. Capillitium formation reaches its highest development in *Geaster hygrometricus*, according to De Bary (1887). In this species the threads arise from hyphae originating in the columella; and after differentiation is complete, a definite skeleton or network is visible. This arrangement, according to De Bary, is somewhat similar to the receptaculum of certain phalloids, and is considered as functioning during spore dispersal. This has led to the opinion that capillitia are effective during spore dispersal. It seems logical, in view of the above similarities in structure and formation, to consider capillitium and the receptaculum homologous structures whose functions have been somewhat modified. Aside from this, little can be found in the gleba upon which relationships can be based.

The sterile bases of different species show different degrees of development. In some species, as in *Calvatia saccata* and *Lycoperdon pyriforme*, the sterile base is relatively large; in other species, as in *Bovista plumbea*, practically no sterile base is developed, while in *Lycoperdon Wrightii* there is a small, poorly developed one. The sterile base, although not as sensitive to environment as the fertile portion, may be somewhat affected by it. Ordinarily the bases do not develop as early as the fertile part; instead, they remain relatively inactive during the early developmental stages. When they begin to differentiate, elongation and increase in diameter take place simultaneously, and by the time of spore formation, the sterile base has reached its specific proportions. According to De Bary (1887), the columella of *Geaster* is really a much reduced sterile base which occupies a relatively small area in the fertile region and often disappears at maturity. From *Calvatia saccata* through *Lycoperdon pyriforme, Lycoperdon pulcherrimum, Lycoperdon Wrightii,* and *Bovista plumbea* there is a gradual reduction in the size of the sterile base until we find it either absent or reduced to the internal columella in the higher Lycoperdaceae as shown by the geasters.

Fruit body modification for spore dispersal varies in different species. In *Calvatia saccata*, with only one peridium, this outer layer usually breaks up into irregular pieces and falls away. Occasionally in this species an apical opening is formed, but this is not characteristic as it is in species of *Lycoperdon, Bovista,* and *Geaster*. Although the details of pore formation are very hard to detect, the rearrangement and thinning out of the hyphae at

the apex were seen to take place in the manner described by Rehsteiner (1892). In *Bovista plumbea* and *Lycoperdon Wrightii* the exoperidium falls away before pore formation takes place, but this is not necessarily true in all species. The highest type of pore formation is seen in the genus *Geaster*, where the pore character is of great taxonomic importance. Calvatias having no pores may, therefore, be considered the simplest, with the lycoperdons and bovistas occupying intermediate positions.

Previous workers have failed to examine rhizomorphs in any great detail. Of the species studied in this paper, *Lycoperdon pyriforme* has the most highly specialized rhizomorph. In this species they are stout, much branched structures. The internal structure strongly resembles that of *Calvatia saccata*, where they are not nearly as strongly developed. The connection of the rhizomorph and fruit body is particularly weak in *Bovista plumbea;* this explains the ease with which these fruit bodies become "tumblers." The other two species treated in this paper are as yet incompletely investigated. The habitat of these fungi usually plays a very important part in determining the nature of the rhizomorph. This is illustrated very well by *Lycoperdon pyriforme*, which is ordinarily collected on wood in some'stage of decay; the rhizomorph in this species sometimes extends more than a foot away from the fruit body. In the ground-inhabiting species such well developed rhizomorphs are seldom found; instead, they are smaller and confined to a more restricted zone in the substratum. In *Geaster*, as described by Rehsteiner (1892), no rhizomorph is developed; instead, the fruit bodies are attached only by weak tufts of mycelium. Considering the growth habits of *Geaster*, this modification seems much to its advantage in allowing the fruit body to be raised up at maturity. This reflexing as well as the ease of detachment from the substratum should greatly aid in spore dispersal. These characters combined with the others mentioned above seem to indicate the advanced position of the geasters in the family Lycoperdaceae.

SUMMARY

1. The detailed morphological development of the following species was studied: (1) *Calvatia saccata*, (2) *Lycoperdon pyriforme*, (3) *Lycoperdon pulcherrimum*, (4) *Lycoperdon Wrightii*, (5) *Bovista plumbea*.

2. The peridium of *Calvatia saccata* was found to consist of only one layer. This finding is at variance with the reports of others who describe a two-layered peridium for this genus.

3. The finding of a definitely one-layered peridium adds another morphological character to those ordinarily accepted for *Calvatia*. This character is sufficient to establish the validity of the genus *Calvatia* as distinct from *Lycoperdon*.

4. The investigated species of *Lycoperdon* all show clearly a two-layered peridium.

5. A modified sterile region was found at the base of *Bovista plumbea;*

the fruit body, with the exception of the peridia, has been considered completely fertile. The very weak connection of this sterile tissue to the rhizomorph explains the ease with which the fruit bodies may be separated from the substratum.

6. The rhizomorphs of the investigated species are not homogeneous throughout; they are composed of two or three distinct layers of hyphae. This depends on the species. Ordinarily there are three layers—a cortical layer, a subcortical layer, and a central core.

7. The tissue of the interior of a fruit body initial is very similar to the interior tissue of the rhizomorph and arises directly from it, and the tissues of the fruit body differentiate from this.

8. The spines, warts, and other exterior markings of the fruit bodies arise simply from the failure of this outer layer to develop as rapidly as the interior. Uneven drying as well as continued internal expansion causes these exterior roughenings to fall off.

9. Cavities arise during differentiation as interspaces between hyphae. This is due to a mechanical splitting rather than to a dissolving of original hyphae.

10. The fertile and sterile regions are very rarely separated by definite morphological structures in the fruit bodies investigated. *Lycoperdon Wrightii* is the most noteworthy exception.

11. Capillitial threads develop from growing hyphae, and serve as reservoirs where waste materials accumulate.

12. There is a remarkable similarity in the differentiation of tissue in the five species included in this paper.

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EXPLANATION OF PLATES

PLATE 29

Fig. 1. Longitudinal section of a rhizomorph, showing the heavy, deeply staining hyphae of the cortex, the parallel subcortical layer and the central core.

Fig. 2. Showing a section of a rhizomorph and the attached young fruit body still appearing as a nodule on the outside of the rhizomorph.

Fig. 3. Later stage of figure 2 showing the heavy, dark threads still adhering to the outer edge of the fruit body. The structure is still homogeneous.

Fig. 4. A group of the peripheral hyphae at the apex which have begun to arrange themselves in the characteristic manner of the peridium.

Fig. 5. The enlarged collar-like region at the point of union of the sterile and fertile regions of the fruit body. Also characteristic arrangement of peripheral hyphae of the base.

Fig. 6. A very young fruit body in which the peridium is well developed and cavities are beginning to appear between loosely interwoven hyphae. Note that the vertical hyphae of the base are still attached to the hyphae of the rhizomorph.

Fig. 7. A segment of the hymenial layer of a cavity, showing the compact arrangement of the hymenium, and the loose arrangement of the subhymenial hyphae. Note the comparatively abrupt connection to the parallel hyphae of the trama.

Fig. 8. A highly magnified cavity showing the relation of the hymenial layer to the parallel tramal hyphae and showing the intercalation of newly formed basidia; the basidia are not mature.

PLATE 30

Fig. 9. A portion of the hyphae between cavities showing the manner in which capillitium is formed from growing hyphae.

Fig. 10. The basal connection of *Bovista plumbea*. The central hyphae are loose, and this accounts for the breaking away of the fruit body in the center.

Fig. 11. A section of the peridia of *Bovista plumbea* showing the tightly woven endoperidium, connected to the pseudoparenchymatous inner layer of the exoperidium. Also note the tangentially parallel arrangement of the outermost hyphae.
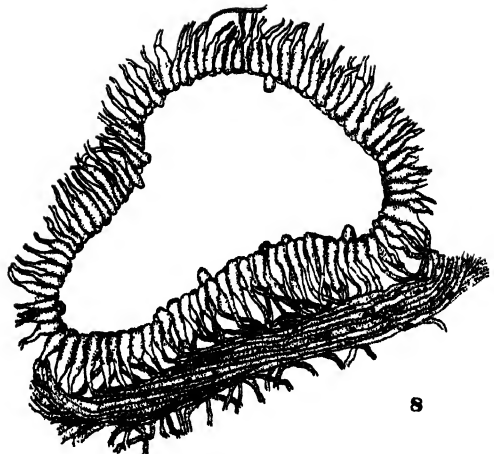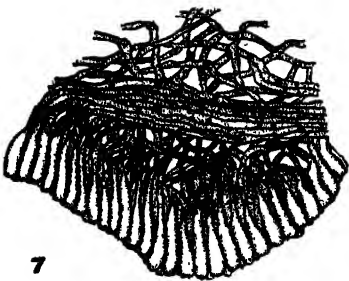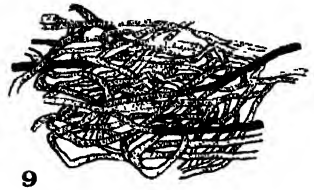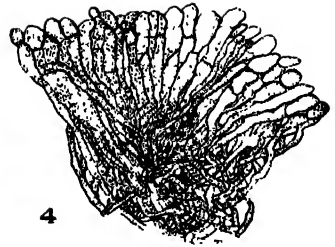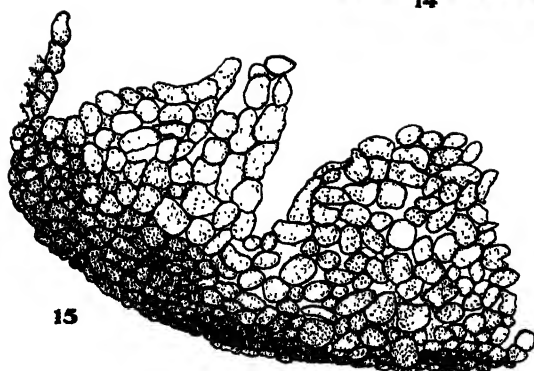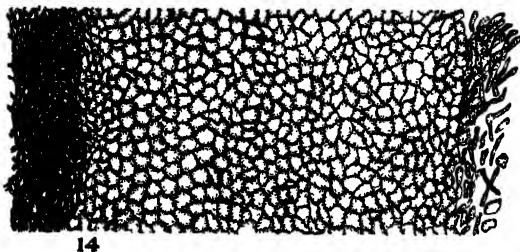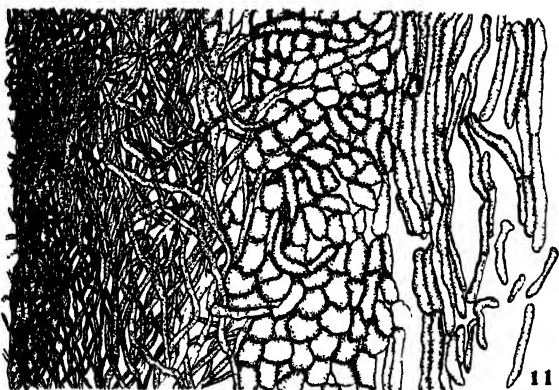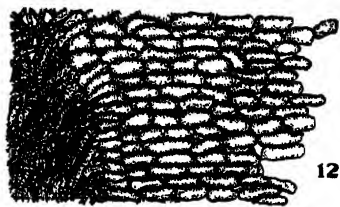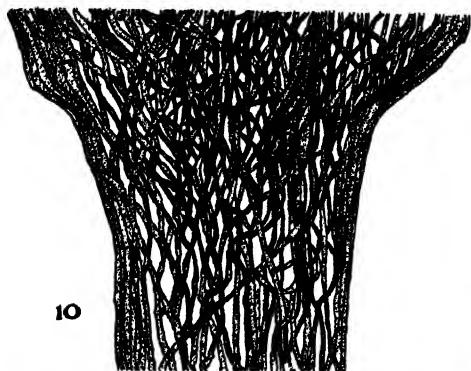
Fig. 12. A small section of the periphery of the sterile base of *Lycoperdon pulcherrimum* showing the parallel arrangement of the outermost hyphae.

Fig. 13. A diagrammatic cross section of a young fruit body of *Lycoperdon Wrightii* showing the relative thickness of the exoperidium.

Fig. 14. A detailed drawing showing the layers of the peridia of *Lycoperdon Wrightii* before the fissuring has begun. Note the thickness of the pseudoparenchymatous layer, and the loose connection of this layer to the endoperidium. This accounts for the splitting here. Compare with *Bovista plumbea*.

Fig. 15. A section of the exoperidium of *Lycoperdon pyriforme*, illustrating the manner in which the exoperidium becomes fissured, and how the outer cells become loosened and eventually fall away.

46

SOME MORPHOLOGICAL AND PHYSICO–CHEMICAL CHANGES ACCOMPANYING PROLIFERATION OF *BRYOPHYLLUM* LEAVES [1]

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INTRODUCTION

The growth of new plants from the notches along the margins of *Bryo-phyllum* leaves may occur while the leaves are attached to the plant, but can be brought about any time by simply removing the leaves from the plant and leaving them exposed to greenhouse conditions. The process will be accelerated if the leaves are placed on a moist surface. This paper deals mainly with the changes that take place in the leaf notches during the various stages of this growth which has been called regeneration by many writers.

Braun (1918), Reed (1923), Howe (1931), and many others have reported regeneration on the intact leaves of healthy *Bryophyllum* plants. Loeb (1918) and Appleman (1918) would account for such growth through some sickness or abnormality of the plants. In a great many cases the meristematic tissue in the notches of the leaves remains dormant until the leaves are removed from the plant. Many explanations have been offered for this dormancy. For example Reed (1923), McIntyre (1918), and Mehrlich (1931) think it is due to a lack of the proper physical and chemical conditions in the leaf for growth; McCallum (1905) and Child and Bellamy (1916) believe that the apical part of a plant through a protoplasmic stimulus inhibits growth from the notches; Fernald (1925) and Child (1921) suggest that apical dominance over foliar shoots is due to a physiological gradient in sap density, electrical potential, and so forth; Loeb (1918–1919a) holds that a specific inhibitory substance produced in the growing part of the plant and carried to other parts by the sap explains the dominance of the apical part of a *Bryophyllum* plant over the rest of the plant; Lamprecht (1918) and Loeb (1915, 1916) account for the dormancy on the basis of a shortage of special growth promoting substance which is being attracted to some other part of the plant; while Fyson and Venkataraman (1921) believe that the supply of water is the limiting factor.

By far the greater part of the work on the proliferation of *Bryophyllum* leaves has been limited to the responses of the plants or leaves to environ-

mental conditions, with considerable speculation as to the internal conditions underlying the responses.   It is the intention of this paper to present the subject more from the point of view of the internal reactions, from four different angles of study: (*a*) morphological, (*b*) physiological, (*c*) microchemical, and (*d*) macrochemical.   In most of the following studies both *Bryophyllum calycinum* and *Bryophyllum crenatum* have been used, since proliferation of the leaves is very similar in both species.   All of the experiments were carried on under greenhouse conditions during the years 1929 to 1932.   More than one hundred plants of each species were used during the study.

MORPHOLOGY OF PROLIFERATION

A knowledge of the development of *Bryophyllum* leaves from a very young stage through maturity and successive stages of shoot and root formation is important in interpreting proliferation and dormancy of foliar buds. This study includes both *Bryophyllum crenatum* and *Bryophyllum calycinum*. The latter is included for comparative purposes only, since it has been described rather thoroughly by Beals, Howe, Naylor, and Yarbrough.   The leaves were collected during March, April, and September.   Those to be studied for various stages of proliferation were placed on moist sand until the desired shoot and root growth had taken place.   No marked seasonal variations were noted in the manner in which they developed.

The notches of very young leaves (about 5 mm. in width) contain a few cells of meristematic tissue which apparently has no connection with the phloem of the adjacent vein (fig. A, B, pl. 31).   At maturity, in both species, this tissue has developed 'in size and number of cells into a well organized primordium (fig. B, pl. 31; G, pl. 32).   This is the stage referred to by Loeb (1915) and Beals (1923) as a bud, and by Naylor (1932) as an embryo.   Attempts to find just where and when cell division takes place have been unsuccessful, both in the ontogeny of the meristematic tissue and in its later development.   The state of development reached by the embryonic tissues in the notches of mature leaves does not appear to be definite.   In some cases for *Bryophyllum calycinum* only a basal portion and stem primordium are evident, while in other cases the stem, leaf, and root primordia and foot, mentioned by Naylor, are present.   In *Bryophyllum crenatum* no root primordia have been found in the foliar buds, only stem and leaf primordia being present.   Later discussion will show that root development is different in the two species during proliferation, of the leaf buds.   This irregularity of development is not strange, since the meristematic tissue in the leaf sinus may or may not pass into dormancy as the leaf matures.   A dormant period beginning in the mature leaf is most common, but the time and state of development reached by the foliar buds are probably controlled by many varying physiological factors.   A study of the development of such buds, after dormancy is broken by removal of the parent leaves from the stem, does not confirm the statement of Beals (1923) that growth in the

notches of the *Bryophyllum calycinum* leaves arises from division of the phloem cells and that the roots develop first. The data do support the reports of Howe (1931), Naylor (1932), and Yarbrough (1932) that the exogenous development of the stem and leaf primordia is first in *Bryophyllum calycinum*. This is even more marked in *B. crenatum* (fig. C, D, pl. 31; H, pl. 32). Early stem growth is more rapid in *Bryophyllum crenatum* than in *B. calycinum* and subsequent root growth slower in the first than in the latter. This has led to the erroneous conclusion by some, who have superficially examined the proliferating leaves of the two species, that *Bryophyllum calycinum* produces roots before shoots.

Root development is endogenous in both species. The roots originate from the basal portion of the stem primordium in *Bryophyllum calycinum* and grow out through the parenchyma tissue of the parent leaf (fig. I, pl. 32). In *B. crenatum* the roots originate in the stem beyond the parent leaf (fig. D, pl. 31). Thus it becomes evident why root primordia are not found in the foliar buds of this species, as stated above. The vascular system of the roots in both species becomes continuous with that of the shoot, which in turn becomes continuous with the adjacent vein of the parent leaf.

This study has revealed the "apex patches" reported by Yarbrough (1932). These meristematic patches are surrounded by veins and are located in the lobes of the leaf. The tissue resembles in some respects that of the foliar buds, but the writer has been unable to discover its significance.

Aside from the anatomical and morphological interest attached to the study of the development of foliar shoots, a problem becomes evident which has not been mentioned by any worker except Yarbrough—namely, why do foliar buds become dormant? This question precedes the one on the breaking of dormancy usually discussed. This paper does not attempt to answer the proposed question, but only suggests that it deserves consideration in the interpretation of data regarding the proliferation of *Bryophyllum* leaves. In view of the above stages of development, it is difficult to imagine how some of the earlier explanations of the dormancy of foliar buds can apply. If there are specific growth inhibitors, why do they fail to inhibit growth of the meristematic tissue in the notches before the leaves mature? If apical dominance is strongest near the apex, how may one explain the beginning of growth in the notches of young leaves in that vicinity which continues until the leaves are mature? If wound hormones and special growth-promoting substances are necessary to start the development of foliar buds into shoots, what caused the development from a few meristematic cells into a rather well organized primordium in the mature leaf notch? To the writer it seems more logical to assume that the production of meristematic tissue in the notches of *Bryophyllum* leaves is hereditary. This development begins in very young leaves and continues through their development to maturity and on to the production of young plants on the margins of the intact leaves, with little or no interruption, unless there develops in the maturing leaf a physical

48

and chemical condition which is not conducive to further cell division and growth.

PHYSIOLOGICAL CHANGES ACCOMPANYING PROLIFERATION

Changes in osmotic value, pH, and total acidity

Fernald (1925) reports that in *Bryophyllum,* in which the apical buds failed to inhibit the growth of the lower ones, the sap concentration in the apical portion did not exceed that in the lower portion. Gustafson (1924–1925) confirms the work of Heyne, Link, Mayer, and Warburg in showing that the hydrogen-ion concentration in *Bryophyllum calycinum* leaves changes diurnally in a manner corresponding approximately to the total acidity changes. He states that the hydrogen-ion concentration increases in the night and decreases during the day, mostly as a result of the influence of light.

In view of the above, it seems worth while to present some data on the changes in pH, total acidity, and osmotic value that take place in the leaves of *Bryophyllum* during proliferation, both in the margin, where growth occurs, and in the central portion. It is reasonable to assume that the acidity and sap concentration of opposite leaves are approximately equal. Thut (1926) has found this to be true in regard to pH. If a comparison is to be made between leaves before and after growth, one of each pair should be left on the plant until the determinations are to be made; otherwise, one can not be sure that any changes found in the proliferating leaves would not have occurred if the leaves had been left on the plant. So, for the following determinations, one leaf from each of a number of pairs was removed and placed on wet sand on the same table with the plants. After the desired period of growth (one day, two days, and so forth), these leaves and the paired ones on the plants were removed, rinsed in distilled water, and dried with a cloth. Marginal strips were trimmed from each leaf sufficiently wide (about .5 cm.) to include the notches. Comparable strips were cut from the centers of the leaves, eliminating large veins. Then the margins and centers of each set were placed in test tubes in an alcohol freezing bath at − 20°C. and frozen 12 or more hours. Since Gustafson reports a diurnal change in acidity, all leaves were collected for freezing at the same time of day, between 6 and 7 p.m. After freezing, the leaves were allowed to thaw at room temperature. The sap was then extracted with a hydraulic press at a pressure of 225 pounds per square inch. The cryoscopic method was used in making the osmotic determinations. The methods of extracting the sap and determining the osmotic values are those used by Meyer (1927). The electrometric method was used in determining the hydrogen-ion concentration. For total acidity determinations, 10 cc. of sap were titrated against a .02 N NaOH, using phenolphthalein as an indicator. The results obtained during June and July are given in tables 1 and 2.

TABLE I.   *Changes in pH, osmotic value, and total acidity of Bryophyllum calycinum leaves during proliferation as compared with paired inactive leaves.   Based on 100 leaves during June and July.*

Groups of paired leaves	Time of growth (days)	pH		O. V. (atmospheres)		Total acidity of 10 cc. sample	
		Center	Margin	Center	Margin	Center	Margin
A	0	3.84	4.27	4.82	3.13	17.9	19.6
	1	3.34	3.49	3.62	3.38	52.0	38.0
B	0	4.80	5.16	3.70	3.90	—	--
	2	5.26	5.35	3.05	4.65	—	—
C	0	4.83	4.83	3.19	4.29	—	—
	4	5.26	5.25	3.20	3.20	—	—
D	0	4.09	4.29	2.77	2.17	45.0	37.0
	6	4.80	4.81	1.80	2.29	27.6	24.4

TABLE 2   *Changes in pH, osmotic value, and total acidity of Bryophyllum crenatum leaves during proliferation as compared with paired inactive leaves.   Based on 100 leaves during July.*

Groups of paired leaves	Time of growth (days)	pH		O. V. (atmospheres)		Total acidity of 10 cc. sample	
		Center	Margin	Center	Margin	Center	Margin
A	0	4.47	4.42	2.65	5.42	8.6	7.6
	1	3.86	3.88	2.77	2.65	19.8	18.0
B	0	4.15	4.33	2.41	1.93	19.6	16.4
	6	4.85	4.56	1.69	3.74	20.4	26.2

The values included in these tables are probably not identical with those existing in the living cells. However, they represent an approximation of living cell conditions. There are some objections to the use of the marginal strips of the leaves as representative of the growing portion, since only a small part of such a strip—namely, the notches—is directly concerned with growth. But the errors would probably be greater, due to the time factor, enzyme action, and so forth, if one attempted to cut out enough material just from the notches for the determinations. Even though the methods are not exact, the figures are useful for comparative purposes.

In tables 1 and 2, the paired leaves (one-half of which were active or proliferating) are grouped together. From these tables several facts are evident. The pH of the margins of the inactive leaves of both species is higher than that of the centers. This was also found to be true under winter conditions. No definite correlation between the osmotic values of the centers

and margins of the non-proliferating leaves was found under summer conditions. but under winter conditions the leaf margins of both species had a much higher osmotic value than the centers.

A close examination of table 1 for *Bryophyllum calycinum* leaves shows that the pH of the margins of the proliferating leaves as compared with the margins of the non-proliferating leaves decreases .78 in 24 hours, and increases .19 in 48 hours, .42 in 4 days, and .52 in 6 days. There are similar changes in the centers of the active leaves as compared with the centers of the dormant leaves. Therefore, the hydrogen-ion concentration increases in both parts of these proliferating leaves during the first day and decreases during the three longer periods. The results show a corresponding increase in total acidity in the proliferating leaves of this species at the end of 24 hours, followed by a decrease at the end of 6 days. Since the notches of the leaf margins are the regions of growth, changes in the leaf margins are probably of greatest importance for consideration. A comparison of the osmotic values in the same table shows that the margins of the proliferating leaves usually have a higher osmotic value than the margins of the paired non-proliferating leaves. During the same periods the centers of the reproducing leaves usually have an osmotic value lower than the centers of the dormant paired leaves.

A study of table 2 for *Bryophyllum crenatum* shows changes in hydrogen-ion concentration, osmotic value, and total acidity similar to those indicated for *Bryophyllum calycinum*. From this it may be concluded that the removal of the leaves from either of these species of *Bryophyllum* plants soon results in increases in pH, total acidity, and osmotic value in such leaf margins. However, it should be noted that while such changes may occur in 24 hours under summer conditions, they may require five or six days in March. But this becomes more understandable when it is known that vegetative reproduction on removed leaves is much slower in the winter time, often requiring twice as long as in the summer time. Whether these seasonal changes are due to internal differences or to light and temperature is not known.

It would be impossible to say from the data at hand whether the above-mentioned physiological changes precede and initiate, accompany, or result from the growth of the dormant meristem in the notches of the leaves. But certainly such an increase in hydrogen ions and total acidity would have considerable effect on protoplasmic viscosity, cell permeability, and enzyme action, which are generally believed to be directly connected with cell division and enlargement. The increase in osmotic value may be explained by an increase in total sugars, as shown by chemical analyses discussed later in the paper. Due to the somewhat inconsistent variation of osmotic values, the writer does not consider them of major importance in explaining the dormancy of foliar buds. A determination of pH, total acidity, and osmotic value for the growing stem tips of both species of *Bryophyllum*, and a comparison with similar determinations made on the leaves of the same plants before and after

growth of the marginal buds gave little or no correlation. It would require many more experiments to determine whether the conditions necessary for the production of shoots on the leaves are the same as for growth at the apex of the plant.

The reasons for an early increase in total acidity and hydrogen-ion concentration followed later by a decrease of both, in proliferating leaves, are not known. Since the leaves were placed with the lower faces down, thus partially blocking the stomatal openings, except on the upper epidermis, it is possible that there was an increased acidity from the accumulation of organic acids and CO_2 due to anaerobic respiration. Similar stomatal closure may result from partial desiccation when the leaves are not placed on a moist surface. The later decreases in acidity may be due to further oxidation of some of the organic acids as a result of increased respiration accompanying growth, or their synthesis into other products as proteins and carbohydrates. Kakesita (1930) has reported regeneration as a result of anaerobic respiration, but Mehrlich (1931) does not confirm his work. Even though intramolecular respiration should be found to bring about physical and chemical changes that result in the proliferation of foliar buds in special cases as above, it is difficult to apply such an explanation to the same growth when it occurs naturally on intact *Bryophyllum* leaves.

Effect of length of day

Braun (1918) reports regeneration on the leaves of *Bryophyllum* plants in the spring. Reed (1923) states that regeneration occurred on plants kept in the dark box for two weeks. McIntyre (1918) says that such growth takes place more profusely in the winter time when growth of the plants is slow. So it seems worth while to report a series of experiments carried on under varying lengths of day. The experiments were run from January 18 until June 1. For short-day conditions, shallow, well ventilated boxes were used and uncovered the desired number of hours each day. For long days, daylight was supplemented in the morning by a 150-watt Mazda lamp which was operated by a Tork clock switch. The periods of lighting used were as follows: complete darkness, 7, 10, and 14 hours. Ten or more plants of each of the two species of *Bryophyllum* were used in each experiment. The plants all averaged about 8 inches in height at the beginning of the experiments.

The most pronounced results were differences in plant growth. The plants in the total darkness became etiolated, and their leaves abscissed without showing any signs of shoot production from their margins. Under the other periods of light there was no growth from foliar buds which one could be sure was not the result of injury or mutilation of the plant. So, under the conditions of the above experiments, retention or the breaking of dormancy of the buds in the notches of intact *Bryophyllum* leaves does not seem to be directly related to length of day. Other plants have been observed during

a period in which the short winter days lengthened naturally into long summer days, without any marked proliferation on the intact leaves of small plants. But from January until early spring large plants of both of the species showed marked growth from the foliar buds. Most of such plants bloomed during this period. Since the breaking of dormancy of the foliar buds sometimes preceded blooming, sometimes followed it, or occurred on plants that never bloomed, no apparent correlation exists between the two phenomena. And since not nearly all of the plants showed the foliar buds breaking dormancy, there seems to be no direct correlation between this process and the length of day.

Effect of humidity and water supply

Several workers have studied the correlation of humidity and water supply with the proliferation of both intact and removed *Bryophyllum* leaves. Loeb (1918), working with *Bryophyllum calycinum,* attributed the greater regeneration on the lower edges of leaves hung in a vertical position in a moist chamber to the collection of sap or water in that portion of the leaves. He makes the statement, " . . . we are justified therefore in assuming that the increase in the content of water in a notch or the starting of a current of water through the notch starts its growth." Reed (1923) reports that leaves on plants regenerate when immersed in water. Fyson and Venkataraman (1921) came to the conclusion that the formation of roots from the notches of leaves depends on the moisture supply and not on the position of the leaf. In contrast, McIntyre (1918) and McCallum (1905) have concluded that water is not of primary importance in the initiation of regeneration.

To check on the above statements, two types of experiments were tried on entire plants which were about one foot tall. First, six plants of each species were kept well watered under bell jars, and aerated once each day for four weeks. Second, six plants of each of the two species were placed under a fine spray of water from March 17 until June 1. The pots were left open, and the plants were kept dripping wet all of the time. Either of the above arrangements should serve to keep the general average water content of the leaves and notches at a maximum without introducing any anaerobic conditions. All of the plants continued to grow well in both conditions, but none showed any signs of foliar shoot development.

Another kind of experiment was tried on the leaves alone. Leaves from both species were placed on moist cotton between large pieces of window glass and aerated daily. In some experiments one of the glasses was replaced by screen wire to hold the leaves in position which eliminated the necessity for aeration. Some of the plates were placed on edge so that the faces of the leaves were vertical. Others were left in a horizontal position. Some of the plates were well watered and others were left dry. The experiments were carried on both in light and in the dark box, being allowed to run until

proliferation was quite evident, which required a period of about 10 days. The percentages of foliar buds that grew in each half of the leaves are given in table 3. The left half was the lower side in the vertical leaves.

TABLE 3. *Percentage of leaf notches that grew under variations of position, water, and light. Based on 50 to 100 leaves in each case. Data obtained for the wet in June and for the dry in August.*

			Percentage of notches proliferating			
			B. *calycinum*		B. *crenatum*	
Environment		Leaf position	Right half	Left half	Right half	Left half
In the light	Wet	Horizontal.	45	38	87	85
		Vertical..........	4	63	92	89
	Dry	Vertical....... ..	90	92	87	87
In the dark	Wet	Vertical	76	74	31	34
	Dry	Vertical	90	90	43	43

The data in table 3 show a decidedly greater percentage of proliferating notches on the lower half of *Bryophyllum calycinum* leaves when in the light on a moist surface with the faces vertical and the midribs horizontal. Such is not the case in the dark. The leaves of this species when grown in this position on a dry surface give a uniform growth of the foliar buds· both in the light and in the dark. From this table it is evident that shoot growth from the notches of *Bryophyllum crenatum* leaves is rather uniform under the various conditions of light, moisture, and position. The results indicate that the collection of water or sap in or on one part of a leaf, due to its position, is not of primary importance in determining the growth of the foliar buds of *Bryophyllum*. However, there is some evidence that light may be a factor.

MICROCHEMICAL STUDY OF PROLIFERATING LEAVES

It seems of first importance to find the initial changes that take place in leaves removed from *Bryophyllum* plants which initiate the growth of the meristematic tissue of the marginal notches of such leaves. A microchemical analysis is one approach to the solution of this problem. This investigation includes only certain changes in enzymes and carbohydrates.

The method used was to remove one leaf from each of a number of pairs and place them on moist sand. After the desired length of time, they were examined and compared with the opposite leaves which had been left on the plants. The reagents were as follows: (1) for oxidase, a 10 per cent solution of guaiaconic acid in alcohol; (2) for catalase, H_2O_2; (3) for diastase, a dilute solution of starch; (4) for starch, I–KI solution and polarized light; and (5) for reducing sugars, Fluckiger reaction (copper tartrate and 20 per cent NaOH), and phenylhydrazine hydrochloride with sodium acetate.

Sections of proliferating leaves when treated with guaiaconic acid showed a decided increase in oxidase, especially in the parenchyma of the notches, as compared with the fresh leaves from the plants. Hydrogen peroxide applied to the leaf sections indicated that catalase was present throughout fresh and proliferating leaves, but more abundant in the latter. No satisfactory microchemical test was found for diastase. A quantitative test was made for it by taking 20 cc. of sap from leaves after grinding them in a mortar and adding 1 cc. of starch solution and a drop of toluene. In every test the diastase activity, as measured by the disappearance of the starch with the I-KI test, was greater for the sap extracted from the proliferating leaves. The foregoing reactions were all stopped by heating the leaves to the boiling point before the tests were made. The above results were found to be equally true for both species of *Bryophyllum,* and confirm the work of McIntyre (1918) on enzymes.

With the aid of I-KI and polarized light, the changes in the distribution of starch in the leaves were followed through various stages of foliar bud growth. From 24 to 48 hours after the removal of the leaves from the plants there is a slight, sometimes complete, disappearance of starch from the leaves. Then there is a gradual increase in starch around the growing points of the notches. This was very marked for *Bryophyllum crenatum.* Even the total starch may increase in such leaves after a time, as will be shown later. In many instances old proliferating leaves, with young marginal shoots two to three inches high still attached, have been found to be gorged with starch. These results are contradictory to those of McIntyre, who reported a complete disappearance of starch from regenerating leaves, starting from the vicinity of the notch and spreading gradually over the entire leaf.

In following the changes in reducing sugars, the osazone method was not found satisfactory. But the Fluckiger reaction showed an increase in reducing sugars, largely glucose, as the length of time of foliar bud growth was prolonged. This increase was first noticeable around the notches and later throughout the leaves, especially along the veins.

MACROCHEMICAL ANALYSES

In order to find some of the major chemical changes that occur, macrochemical analyses were made of *Bryophyllum calycinum* leaves before and after proliferation.[2] For these analyses paired leaves were again used, one of each pair being used fresh while the opposite of each pair were placed on moist sand and allowed to reproduce vegetatively from the notches from March 26 to April 2 before being analyzed. All of the determinations were made in duplicate and with checks. The fresh weight of the leaves analyzed immediately after removal from the plants was 100 g., while the fresh weight

[2] The methods of analysis are those listed in: "An introduction to phytochemical research," by R. C. Burrel, Ph.D., Ohio State University. 1931. Published by the author.

of the leaves that were allowed to grow for seven days was 103.1 g. The fresh weight of the latter after one week of growth was 94.98 g. All of the percentages in table 4 were calculated on the basis of the fresh weight of the leaves at the time they were removed from the plants.

From the above statements in regard to the fresh weights and from the data in table 4, it can be seen that during proliferation of the removed leaves both the fresh and dry weights decreased. But if the percentage of dry matter after growth is calculated on the basis of the fresh weight at that time, it will be found to be about the same as for the fresh leaves. Therefore these data do not support the view that an increase in the water content of the leaves is of major importance in breaking the dormancy of the foliar buds.

It is of further interest to note that although the total solids decreased during growth, the percentage of solids in the alcoholic extract increased. The increase in total sugars during proliferation, given in table 4, may

TABLE 4.   *Polysaccharides expressed as glucose.   Figures are in percentage of fresh weight*

Materials	Residue				Extract								
	Total solids	Total nitrogen	Starch	Total polysaccharides	Total solids	Total nitrogen	Amino acid nitrogen	Ammonia nitrogen	Amide nitrogen	Nitrate nitrogen	Reducing sugars	Total sugars	Sucrose
Fresh leaves...	7.96	.03	.76	1.46	1.55	.02	.008	.0006	.00	.003	.12	.18	.06
Proliferating leaves......	6.44	.02	.36	0.90	2.02	.02	.01	.0005	.0003	.004	.50	.62	.12

explain the increase in osmotic value noted earlier in this paper. Micro-chemical analyses have shown that there is an increase in starch in certain regions of the leaves after removal from the plant. These analyses have given a decrease in total starch for similar leaves. But it should be mentioned that analyses of a second set of leaves, in the same manner as given above, gave similar results to those in table 4 in every respect except for starch, which showed an increase in the proliferating leaves. So it seems that the starch content of such leaves may or may not increase generally as well as locally. Mehrlich (1931) states that the amount of nitrogen may be greater or less under different conditions in active leaves than in dormant leaves. The results in table 4 indicate that this is probably true, but that the combinations of nitrogen may vary greatly. The two different forms of chemical analyses used show that there is plenty of food present in fresh *Bryophyllum* leaves for growth. Therefore the dormancy of foliar buds cannot be explained on the basis of food shortage.

SUMMARY

1. Foliar shoots in the notches of *Bryophyllum crenatum* and *B. caly-cinum* leaves grow from rather definite primordial tissues which begin to develop in the notches of very young leaves.

2. Stem and leaf primordia begin to grow first in proliferation and are exogenous in both species. Root development is endogenous in both, but the point of origin is different in the two species.

3. The vascular system of the foliar shoot becomes continuous with that of the parent leaf.

4. In both species the hydrogen-ion concentration and total acidity of the margins of the proliferating leaves increase during the first few days and then decrease.

5. The osmotic value of the margins of proliferating leaves is, in general, higher than in the margins of the paired inactive leaves.

6. There seems to be no direct correlation between the retention or breaking of dormancy of foliar buds on intact leaves and length of day.

7. Water and leaf position are not of primary importance in determining the dormancy of foliar buds.

8. The following chemical changes take place during proliferation of *Bryophyllum* leaves: (*a*) accumulation of starch around the growing foliar buds; (*b*) increase in reducing sugars around the foliar buds; (*c*) increase in total sugars and sucrose in the entire leaf; (*d*) decrease in both fresh and dry weight of entire leaf; (*e*) decrease in total polysaccharides in the entire leaf; (*f*) increase in amino nitrogen, amide nitrogen, and nitrate nitrogen in the entire leaf; (*g*) increase in oxidase, catalase, and diastase around the foliar buds.

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EXPLANATION OF PLATES

PLATE 31

Bryophyllum crenatum

Fig. A.   Cross section of a young leaf .5 cm. in width, cut parallel to the face of the leaf; *n,* the notch; *em,* the first few cells of the meristematic tissue in a notch. × 280.

Fig. B.   Cross section of a mature leaf cut parallel to the face of the leaf, showing notch, foliar embryo, and adjacent vein.   × 100.

Fig. C.   Cross section of proliferating leaf at right angle with the face of the leaf through a notch, showing the development of the foliar shoot during one day; *pr,* parent leaf.   × 100.

Fig. D.   Cross section of a proliferating leaf at right angle with the face of the leaf, showing five days' development of the foliar shoot, *sh.*   × 100.

Fig. E.   Cross section of a proliferating leaf through a notch at right angle with the face of the leaf, showing nine days' growth of the shoot, *sh;* the origin of the root, *r,* beyond the parent leaf, *pr.*   × 100.

PLATE 32

Bryophyllum calycinum

Fig. F.   Cross section of a young leaf .5 cm. in width, cut parallel to the face of the leaf; showing the notch, *n;* and the first few cells of the meristematic tissue, *em.*   × 250.

Fig. G.   Cross section of a mature leaf, cut parallel to the face of the leaf; showing the notch, foliar bud, and adjacent vein.   × 100.

Fig. H.   Cross section through a proliferating notch at a right angle with the face of the leaf; showing parent leaf, *pr;* root primordia, *r;* and leaf primordia, *x;* after one day of growth.   × 100.

Fig. I.   Cross section through a proliferating leaf notch at a right angle with the face of the leaf; showing parent leaf, *pr;* shoot, *sh;* and root, *r,* growing out through the parent leaf; after eight days' growth.   × 100.
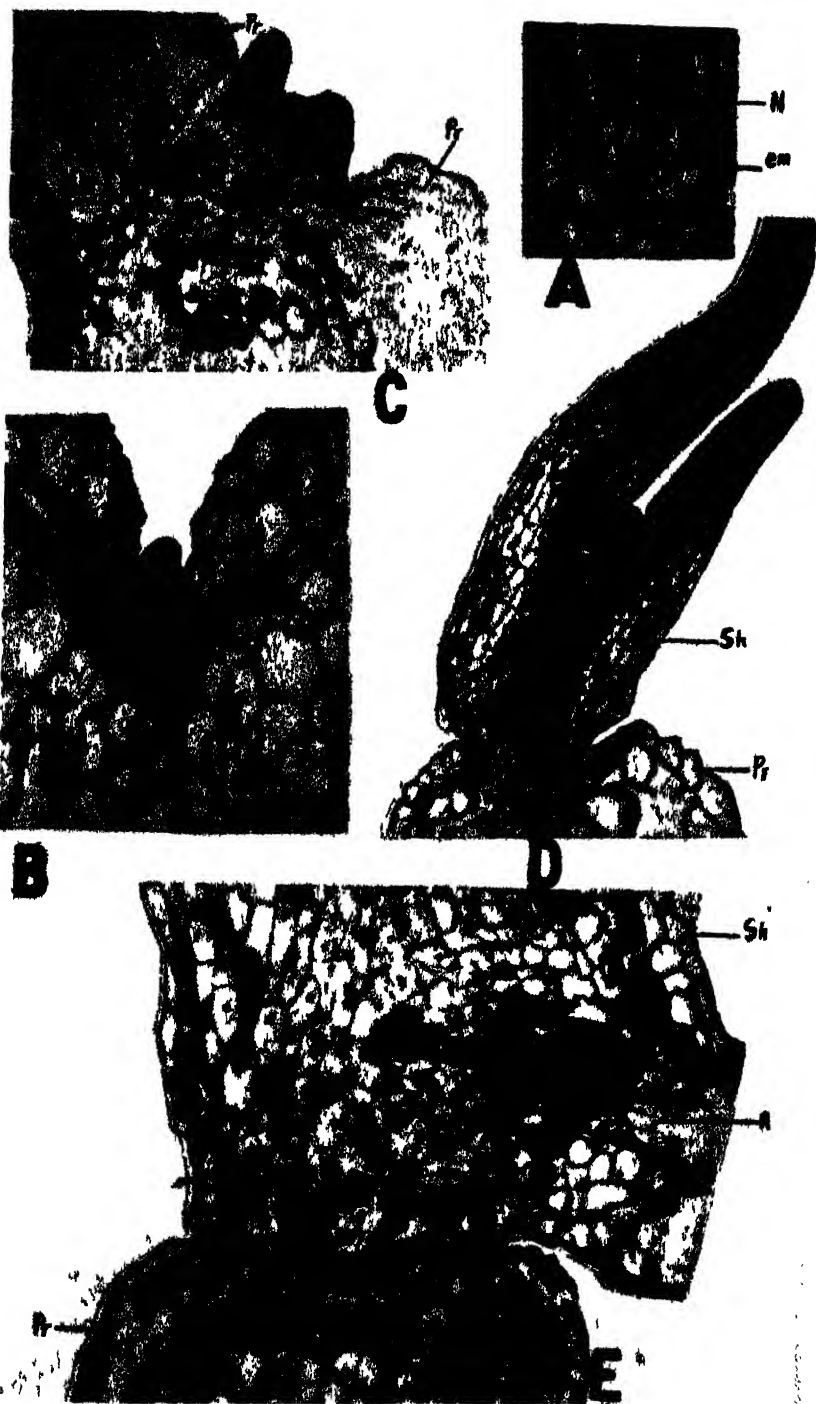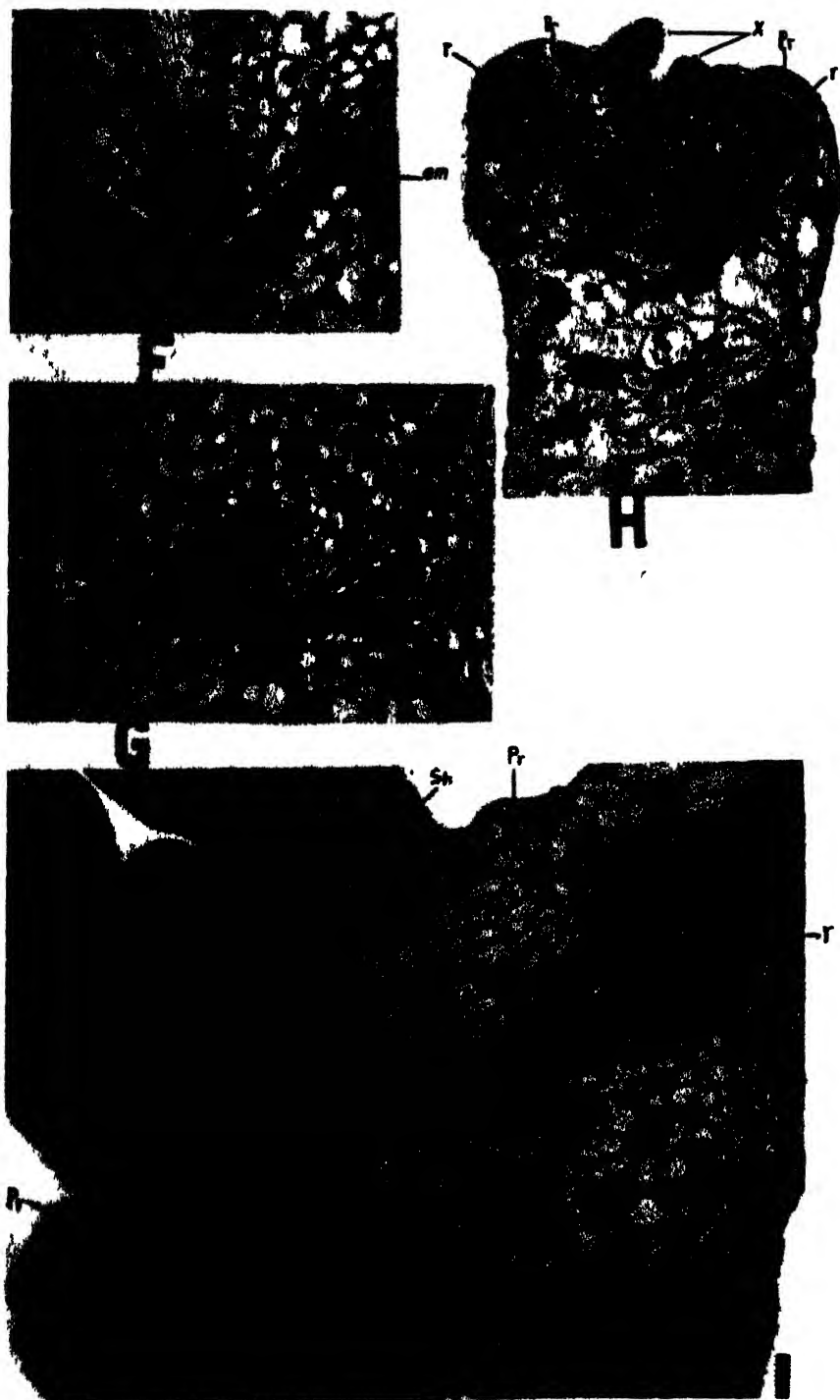
FREELAND   BRYOPHYLLUM

THE INTAKE OF WATER THROUGH DEAD ROOT SYSTEMS AND ITS RELATION TO THE PROBLEM OF ABSORPTION BY TRANSPIRING PLANTS

PAUL J. KRAMER

INTRODUCTION

In a recent paper the writer reported the results of certain experiments which indicated that transpiring plants can absorb water through root systems which have been killed (Kramer, 1932). It is evident that none of those processes which are dependent on the presence of living cells in the roots can be effective in bringing about the intake of water through dead root systems. Any absorption of water occurring under these conditions must therefore be brought about by forces originating elsewhere than in the roots. These facts necessitate a reconsideration of the traditional view that the intake of water is brought about by osmotic and imbibitional forces developed in the living cells of the roots. This paper presents the results of a further investigation of the absorption of water through dead root systems.

The ability of plants to absorb water through dead root systems was investigated many years ago, but the results of these early investigations seem to have been largely overlooked by more recent workers. Strasburger (1891) summarized the results of three such studies, and the following facts are for the most part taken from this discussion. A. Hansen (1884–1885) killed the roots of several species of potted plants by heating the soil in the pots to a temperature of 65°–70°C. This temperature was maintained for two hours. Other plants were removed from the soil, placed in water at 70°–80°C. for one-half hour, and then placed in fresh water. Plants of several other species grown in water cultures were treated in the same manner. In every experiment the tops remained fresh and unwilted for several days after the roots were killed. Considerable quantities of water were absorbed through the dead roots, though the amount was less than that absorbed by plants with living roots. Hansen in each case determined by direct observation that the roots were actually dead. He regarded these experiments as affording clear evidence that the assistance of what he termed "a living root force" or "root pressure" was not necessary to bring about absorption by transpiring plants. He concluded that water moved through the cell walls by imbibition, a conclusion which was criticized by Strasburger.

Janse (1887) repeated some of Hansen's experiments with similar results.

He further reported that if root systems were placed in a partial vacuum little absorption occurred. This led him to conclude that atmospheric pressure plays a part in absorption. Boehm (1889) likewise investigated the absorption of water through dead roots. He found, in contradiction to Janse. that the atmospheric pressure on the roots could be decreased nearly to the tension of water vapor without materially affecting the rate of transpiration and hence the rate of absorption. He also reported that no important difference existed between the transpiration rates of shaded plants with living and with dead root systems. Boehm concluded that the absorption of water is not brought about by atmospheric pressure or osmosis, but by capillarity.

Strasburger did not accept the explanations of absorption offered by Hansen, Janse, or Boehm, but he fully appreciated the significance of the discovery that the intake of water may occur through dead roots. He stated that this indicated beyond any doubt that a " sucking action " is exerted by the water-conducting vessels on their surroundings which results in their becoming filled with water. He termed this " tracheal suction " and believed it to be similar in origin and nature to the forces bringing about the upward movement of water in the conducting vessels of the stem. Strasburger believed this " tracheal suction " was developed independently of any activity of the living cells of the roots. However, he considered that the experimental evidence available would not permit the view that the rôle of the living cells in absorption was an entirely passive one. In living roots he assumed that the " tracheal suction " caused a withdrawal of water from the cells surrounding the xylem vessels, disturbing the osmotic equilibrium of the cortical cells and resulting in an osmotic movement of water from the epidermis across the cortex toward the xylem. The behavior of dead roots was compared to that of an imbibing sponge, water entering from the soil as it is removed by the " tracheal suction " of the conducting vessels.

Strasburger realized that what he termed " tracheal suction " was inoperative at times when root pressure and bleeding occurred. However, he believed that the amount of water forced into the xylem vessels at such times was quite small as compared with the amount absorbed due to tracheal suction.

If one makes allowance for certain changes in terminology and for the fact that Strasburger knew nothing about the cohesive force of water, his discussion of absorption is far in advance of that of any of his contemporaries, or indeed of many present-day discussions of this problem.

The only further experimental work was that of Watson (1894) and Darwin and Acton (1895), who reported that several species of plants were able to absorb water through dead root systems. However, neither attached any special significance to this fact nor suggested that it had any bearing on an explanation of the absorption process.

EXPERIMENTS AND RESULTS

Experiments with entire plants

Several dozen plants belonging to seven different species were employed in these experiments. The plants were grown in a rather sandy loam soil in ordinary 10-cm. clay pots. The root systems were killed by immersion in hot water until a soil temperature of at least 60°C. was attained throughout the soil mass in each pot. During this process the stems were usually killed for a distance of one to two centimeters above the surface of the soil. An attempt was made to employ ether to kill the roots. The pots were sealed in a metal shell containing ether. in such a manner that the tops projected outside and were not exposed to the ether. The roots were killed, but sufficient ether often diffused up the stems to kill the stems and lower leaves to a height of ten or fifteen centimeters above the soil; so this method was abandoned. The roots of all plants were examined at the end of each experiment to make certain that they had actually been killed.

In experiments with sunflowers the lowermost leaves usually began to turn yellow two to three days after the roots were killed. In some cases small dark-colored areas of dead tissue appeared, mostly along the margins of the leaves. Usually the leaves exhibited no indications of wilting until after the change in color began. The leaves at higher levels were progressively affected until at the end of approximately a week only the uppermost leaves remained green. By this time the lower leaves were often brown and dry. In most experiments with sunflower plants the uppermost leaves remained alive and unwilted for about ten days. Tomato plants behaved in a very similar manner, except that the leaves died more slowly and the upper leaves were sometimes in good condition two weeks after the roots were killed. In all cases the soil containing the plants with dead root systems was kept well watered. Check plants with living roots in unwatered soil usually completely wilted in two to three days. Both tomato and sunflower plants with dead roots exhibited the normal response to very low concentrations of illuminating gas, showing a marked epinastic drooping of the leaves within a few hours after exposure.

Tobacco plants often exhibited yellowing and wilting of the lower leaves within forty-eight hours after the roots were killed, and all of the leaves were usually affected within a week. Cotton plants behaved in a very similar manner. Unwatered plants of both species with living roots wilted in twenty-four hours.

When the roots of two-year-old seedlings of yellow poplar (*Liriodendron tulipifera*) were killed by heat, brown areas often appeared along the margins of the leaves within forty-eight hours, and the leaves were usually somewhat wilted in three days. In nearly all cases the seedlings were dead in a week.

The control plants employed in this and the following experiments were removed from the soil and their roots suspended in a flask over a small

quantity of water. By thus keeping the roots in a saturated atmosphere, no water was lost from them nor was any appreciable quantity absorbed. When the roots of six-months-old seedlings of red oak (*Quercus borealis*) were killed, small dead areas appeared in most of the leaves in three to four days, the leaves began to wilt, and were dead in a week. Control plants were completely wilted in two days. Seedlings [1] of loblolly pine (*Pinus taeda*) remained uninjured for two weeks or more after their roots were killed. Control plants treated in the same manner as the yellow poplar and red oak checks exhibited definite injury in four to five days in the form of curled, twisted, and browned needles.

Comparison of the behavior of the controls with plants the roots of which had been killed indicated that in every case appreciable quantities of water must have been absorbed through the dead roots. The actual amount absorbed was determined by studying the relative transpiration rates of a number of plants with living and with dead root systems.

Although the variations in the behavior of individual plants were rather large, the general trend of the results was quite uniform. In experiments with sunflower, tomato, and tobacco plants it was found that in most cases during the first twenty-four hours after the roots were killed the plants with dead roots transpired only about one-half as much water per unit of leaf area as the plants with living roots. An exception was found in one series of sunflower plants in which during the first twenty-four hours the plants with dead roots lost slightly more water than the plants with living roots. In all experiments the transpiration rate progressively decreased during each successive day until it became almost negligible by the time the uppermost leaves began to wilt.

The results of these experiments leave no doubt that considerable quantities of water are absorbed through dead roots. However, the decreasing transpiration rate and relatively short life of the plants after their roots were killed threw some doubt on the efficiency of dead roots as absorbing organs. An investigation was therefore conducted in order to determine whether these occurrences resulted from inability to absorb sufficient water through dead roots or from other causes.

A comparison of the amounts of water which could be forced through stem segments of equal length taken from plants with dead and with living roots showed that much less water could be forced through the stems of plants with dead roots. In sunflower and tobacco it was found that only one-third to one-half as much water could be forced through stem segments taken from plants twenty-four hours after the roots were killed as through segments of equal length from plants with living roots. In many experiments no water could be forced through stem segments cut three days after the

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[1] Most of the tree seedlings employed in these experiments were secured through the kind coöperation of the North Carolina State Department of Conservation and Development and C. F. Korstian, Director of the Duke Forest.

roots were killed, and not over ten per cent of the normal amount was ever transmitted. Similar tests were made on stem segments taken from plants of tomato, cotton, yellow poplar, and loblolly pine three or four days after the roots were killed, with the same results. The segments were ten or fifteen centimeters in length. Comparisons were always made between segments of equal length, and the same pressure and time intervals were employed. A pressure of approximately one atmosphere was applied for twenty or thirty minutes.

Microscopic examination of the xylem vessels revealed that many were plugged by deposits of a brown material which has been described by Overton (1911) and others as a "gum." The heaviest deposits of this occurred just above the killed tissue, and it was found in decreasing quantities for twenty or thirty centimeters up the stem. Within two or three days after the roots were killed, most of the vessels in the lower part of the stem were completely filled, while at higher levels fewer vessels were filled and in many cases deposits existed only on the walls, leaving the cavities empty. Within twenty-four to forty-eight hours after the roots were killed, the xylem was noticeably stained by the gum. In some cases the movement of water was hindered before the xylem showed any evidence of staining.

Experiments with decapitated plants

Since the rapid plugging of the water-conducting vessels and consequent death of the tops soon brought experiments with entire plants to an end, certain experiments were performed with decapitated plants. In these the conditions existing in the water-conducting vessels of a transpiring plant were partially duplicated by attaching a vacuum pump to the cut stems. The stems were cut off three or four centimeters above the surface of the soil and pieces of glass tubing attached to them by means of short pieces of rubber tubing. These unions were made air- and water-tight by a coating of paraffin. By the use of glass T-tubes and rubber tubing six plants could be connected to the vacuum pump at one time. A good filter pump or vacuum pump reduced the pressure on the ends of the stems to one or two centimeters of mercury, but of course a negative pressure could not be produced by this method.

Loblolly pine, yellow poplar, and cotton plants were employed in these experiments. The amount of water exuding from the cut stems due to a positive pressure or "root pressure" was first determined for a twelve-hour period. The plants were then connected to a vacuum pump, and the amount of water exuding under reduced pressure was ascertained for a similar period of time. The roots were then killed by the use of hot water or ether, and the amount of water secured under suction was again determined for a twelve-hour period. The difficulties encountered in the use of ether to kill root systems with attached tops naturally did not arise with decapitated plants.

In moist soil, cotton plants always exhibited some exudation of water.

When these plants were attached to a vacuum pump, four or five times as much water exuded under the reduced pressure as had exuded during the same period of time due to "root pressure" alone. After the roots were killed, exudation ceased until the plants were again attached to the vacuum pump. Then fifty or more times as much water was secured as had been secured by suction on the same root systems while they were alive. Yellow poplar seedlings behaved in a very similar manner, though the increases secured by the application of suction were much smaller than in the case of cotton. The behavior of the loblolly pine seedlings was quite different. No exudation of water occurred from the living root systems either with or without suction, but considerable quantities were secured under suction after the roots were killed.

Experiments with cotton and loblolly pine root systems indicated that under suction considerable water was absorbed through them three to four weeks after they were killed. It seems probable that water may be absorbed through dead root systems until they are destroyed by decay. Two weeks after they were decapitated, no water could be secured from living root systems, though they remained alive for at least three weeks. In all of these experiments the soil was kept practically saturated with water.

A number of experiments were performed with root systems growing in a relatively dry soil in order to determine whether or not absorption through dead root systems will also occur in soils with a low moisture content. The soil in which the cotton and sunflower plants employed in these experiments were grown was carefully mixed and sifted to assure a uniform composition. It was a sandy loam having a wilting coefficient of about 3.5 per cent as determined experimentally with sunflower and oat seedlings. This gave it a moisture-equivalent value of about 6.4 per cent as calculated from the wilting coefficient according to the formula of Briggs and Shantz (1912). The moisture equivalent was determined experimentally to be about 13 per cent. The moisture content of the soil was kept slightly above the moisture equivalent while the plants were growing. The plants were grown to an age of six to eight weeks, by which time the roots were well distributed through the soil mass in each pot.

The tops were removed and glass tubing was attached to the stems as previously described. It was found that in most cases no exudation of water occurred, but on the contrary, water often passed into the root systems through the cut stems. The root systems were killed by sealing the pots in metal shells containing 15 to 20 ml. of ether for about eighteen hours. Ether was used to obviate the saturation of the soil resulting from killing the roots with hot water.

Both living and dead root systems were attached to a vacuum pump until no appreciable quantities of water passed through them into the attached tubes. As this point was reached, the amount of air entering through the roots increased, resulting in violent bubbling in the tubes, and they were

then disconnected. Samples of soil were then taken from the region of maximum root distribution in each pot, dried, and the moisture content was determined. The moisture content of the soil containing dead root systems was found to be practically the same as that of the soil containing living root systems. In both cases it was 6 to 7 per cent, or approximately equal to the calculated moisture equivalent, but only about half the observed moisture equivalent.

<div align="center">DISCUSSION</div>

It seems quite certain that transpiring plants may absorb sufficient water through dead roots to remain alive and unwilted for several days after the roots have been killed. This conclusion is based on experiments by the workers previously cited as well as on those of the writer. In every experiment the plants with dead roots in well-watered soil lived longer than plants with living roots which were in dry soil or which were removed from the soil. This was true of both herbaceous and woody plants and gymnosperms as well as angiosperms. Although the experimental methods employed permitted only the use of small potted plants, it seems very probable that under similar conditions larger specimens would behave in the same manner.

The injury to the leaves, stoppage of the xylem, and premature death of plants after their roots were killed seem to have been due to the effects of materials escaping from the dead cells of the roots. Dixon (1914), Overton (1911), and others reported similar injury and death of the leaves following the killing of portions of the stem and ascribed it to the deleterious effects of substances escaping from the cells in the killed portion of the stem. The initial injury to the leaves could scarcely have been due to desiccation because discolored and dead areas often appeared before the leaves showed any evidence of wilting. The appearance of these leaves was also noticeably different from that of leaves on plants dying from desiccation. In the latter case the leaves usually wilt and die without losing their green color. This, however, is not always true of tough, leathery leaves which may turn brown and die from desiccation without exhibiting any visible evidence of wilting.

According to Higgins (1919), in most vascular plants deposits of gum may be found in the tissue adjoining areas killed by fungi or poisons or disturbed by mechanical injury. He believed that the gum was produced by the action of pectin-dissolving enzymes and that the formation of these enzymes was stimulated by the injury and death of the cells. According to Dixon (1914), Weber and Janse both reported that the plugging of the vessels and resistance to flow was greatest at the border of the dead tissue, and this was found to be true in the present experiments. Although in some cases no water could be forced through the stems under one atmosphere of pressure, it is possible that since tensions of several atmospheres are frequently developed in transpiring plants some water might be pulled through in spite of the plugging. However, the amount would be much less than that normally passing through. The reduction in leaf area and increased resistance

to the movement of water through the stem seem to be the principal causes of the decrease in transpiration after the roots are killed.

The reduction which was found to occur immediately after the roots were killed may have been due to the physiological shock of the killing process. Many of the stomata of sunflower plants were found to be closed a half-hour after the plants were removed from hot water, and six hours later a large number were still closed. Twenty-four hours later the normal number seemed to be open. However, by this time the xylem was probably already partly plugged.

It was always found that water passed in more rapidly through dead root systems than through living root systems when a vacuum pump was attached to the cut stems. It appears that living roots offer more resistance to the inflow of water than do dead roots. It therefore would not have been surprising if the transpiration rate had been increased at least for a few hours after the roots were killed as a result of the increased ease of absorption. In only one experiment was there any indication of such an increase, and as only about two dozen plants were employed in this experiment, the exceptional results may have been accidental. The transpiration rate of individual plants varies so much from day to day that reliable results can therefore be assured only by the use of large numbers of plants. It appears that any increase in transpiration which might result from the decreased resistance to the movement of water into the vessels of the roots is usually more than balanced by the decrease due to the physiological shock of killing the roots.

The results obtained by attaching a vacuum pump to the root systems of decapitated cotton and yellow poplar plants were in general similar to those secured with sunflowers, tomatoes, and several other species in an earlier investigation (Kramer, 1932). Cotton plants were found to be unusually well adapted to use in these experiments, as the woody stems increased the ease of making and maintaining tight connections to the glass tubing. Much less air entered through the roots of cotton than through the roots of some other species, particularly yellow poplar. A number of yellow poplar seedlings were discarded because large quantities of air entered through the roots, resulting in violent bubbling in the attached tubes. Some bubbling occurred at all times, probably being largely due to air coming out of solution under the reduced pressure existing in the tubes.

The failure to secure any water from living root systems of loblolly pine agreed with the results obtained with Norway spruce in the first investigation. Farmer (1918) reported that there was a much greater resistance to the flow of water in stems of evergreens than in stems of deciduous species. It may be that the pressure gradient developed under suction is not great enough to overcome the resistance in conifers. In all other species studied, the inflow of water was much greater through dead than through living roots. It is probable that this results from a decrease in the resistance to water movement from the periphery to the conducting

vessels of the roots due to the destruction of the protoplasts and their differentially permeable membranes.

An interesting fact brought out by the studies on root systems in soils with a moisture content near or below the moisture equivalent was the existence of a considerable saturation deficit in the roots of many of the plants. This existed even though the plants had stood in a cool, shady place for several hours before being decapitated. Exudation from the cut stems occurred in only a few cases, and both cotton and sunflower root systems frequently absorbed 3 or 4 ml. of water through the cut surfaces of the stems in a twelve-hour period. Kennedy and Crafts (1927) reported the existence of saturation deficits in root systems which seemed to be largely determined by the rate of transpiration prior to cutting the stems. They found that in plants with low transpiration rates the saturation deficit varied inversely with the soil moisture.

It appears that when living and dead root systems are attached to a vacuum pump both are able to reduce the moisture content of the soil to approximately the same extent. In these experiments both sunflower and cotton root systems under suction reduced the soil moisture to 6–7 per cent, which is approximately equal to the moisture equivalent as calculated from the observed wilting coefficient, but is equal to only about half of the observed value for the moisture equivalent. Veihmeyer and Hendrickson (1928) have reported similar discrepancies between the observed and calculated moisture equivalent.

As the moisture content of the soil is reduced below the moisture equivalent, it appears that the films of water surrounding the soil particles become so thin that the movement of water from particle to particle is very slow. Therefore, only the water in the near vicinity of the roots is immediately available to them. It therefore seems unlikely that the soil moisture could be reduced appreciably below the moisture equivalent by dead roots. Living root systems, however, are continually being brought in contact with additional moisture supplies by the growth of the root system, and are able therefore to reduce the soil moisture considerably below the moisture equivalent. Living root systems attached to a vacuum pump probably could not do this. The force bringing about the intake of water in this case is equal to the difference between the pressure inside and outside the roots, which is slightly less than one atmosphere, while the forces with which the water is held to the soil particles are probably greater than this when the moisture content of the soil approaches the wilting coefficient (Shull, 1916).

The absorption of solutes is doubtless considerably affected by the death of the roots. As Strasburger (1891) pointed out, the living cells which separate the xylem from the surface of the root have a marked effect on the entrance of solutes into the plant. Strasburger found that when the differentially permeable membranes were destroyed by killing the roots any kind of solute present in a solution surrounding the dead roots was readily

absorbed.   For instance, he stated that if the roots were killed by immersion in a copper sulphate solution not only was it absorbed in considerable quantities and killed the cells with which it came in contact, but other substances which were then made available were absorbed in greater quantities than through living roots.   It is generally considered that the absorption of minerals is not directly proportional to the absorption of water.   However, it seems probable that the absorption of minerals through dead roots would be approximately proportional to the quantity of water absorbed.

The results of these experiments must be taken into consideration in the formulation of any satisfactory explanation of the mechanism by which the intake of water is brought about in transpiring plants.   The current explanations of this process emphasize the importance of "root pressure," unidirectional secretion, suction tension gradients, electro-endosmosis, and other processes which are dependent on or result from the activity of the living cells of the roots.   Absorption of this type has been termed "active absorption" by Renner (1915) because the living cells of the roots play an essential part in the process.   However, it has been demonstrated that the intake of water may readily take place through dead root systems where none of these processes are operative.   It therefore appears likely that the importance of the osmotic and imbibitional forces of the living cells of the roots in the absorption of water by transpiring plants has been greatly over-emphasized.

It is evident that the forces bringing about absorption in plants with dead roots originate in the tops rather than in the roots.   It seems probable that in most freely transpiring plants, and perhaps even during periods of reduced transpiration, absorption is brought about as a result of the negative pressure or tension developed in the hydrostatic system by the removal of water in transpiration.   Shull (1924) suggested that this tension may be converted into osmotic and imbibitional forces in the cells of the roots.   This might occur in living roots, but could scarcely occur in dead roots where the osmotic membranes have been destroyed and the imbibing materials of the cells altered.   Livingston (1927) suggested that the inflow of water occurs because a gradient of decreasing pressure is produced between the surface of the root and the water-conducting vessels by the tension or negative pressure in the hydrostatic system.   In experiments where a vacuum pump was attached to the root systems, a gradient of one atmosphere was developed; but in rapidly transpiring plants gradients of many atmospheres occur, and the intake of water is doubtless proportionally increased.   Absorption under such conditions is similar, at least so far as the rôle of the roots is concerned, to what has been termed "passive absorption" by Renner (1915) because the roots act merely as absorbing surfaces.

SUMMARY

1. Many of the current explanations of absorption emphasize the importance of "root pressure," unidirectional secretion, suction tension gradients, electro-endosmosis, and other processes occurring only in the presence of living cells in the roots. If such processes play an important rôle in the intake of water, absorption should be greatly reduced by killing the roots and stopping these processes.

2. It was found that transpiring woody and herbaceous plants would remain alive and unwilted for several days after their roots had been killed. In such experiments considerable quantities of water were absorbed from the soil through the dead root systems. The results of these experiments clearly demonstrated that absorption is not stopped by the death of the roots.

3. In most of these experiments the transpiration rate decreased rapidly after the roots were killed. This apparently was due to leaf injury and to the plugging of the xylem by deposits of gum, both probably resulting from the action of substances escaping from the dead cells in the roots.

4. Results of experiments in which a vacuum pump was attached to the stems of decapitated plants indicated that water passed in more rapidly from the soil through dead than through living root systems. The reduction of the moisture content of the soil brought about by living and by dead root systems attached to a vacuum pump was approximately the same.

5. Since in these experiments water was so readily absorbed through dead roots, it seems that the importance of the rôle played by the living cells of the root in absorption has been greatly over-emphasized.

6. It appears that the intake of water by transpiring plants is due largely to the tension or negative pressure developed in the hydrostatic system by the removal of water in transpiration and other processes. This tension produces a gradient of decreasing pressure between the water in the soil and the water in the xylem vessels, and as a result water moves from the soil into the roots.

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MEIOSIS IN *DIGITALIS FERRUGINEA* WITH SPECIAL REFERENCE TO THE ANACHROMATIC AND CATACHROMATIC PROCESSES [1]

SARAH FRANCES WENTZEL

INTRODUCTION

Denniston (1913) reported an investigation of the mitotic process in *Gentiana procera*. His results showed that the chromosomes of this species are very small and that they retain their visible individuality for a much longer period than larger chromosomes do. The prophase was the only stage in which the chromosome's individuality was said to be lost. Eichhorn (1930) also worked with the mitotic process of small chromosomes, using for his material several species of squash. During the entire process the individual chromosomes remain visible, but Eichhorn speaks of the prophase bodies as prochromosomes and not true chromosomes. Yasui (1911), who studied the life history of *Salvinia natans*, saw nothing unusual in the behavior of the chromosomes in the microsporocytes. De Litardiere (1921), several years later, also worked with *Salvinia natans* and *Azolla caroliniana*. According to this author, the chromosomes in these species are extremely small, ranging from 0.5 to 1.5 microns in length. He noticed that those species of ferns with extremely small chromosomes exhibited a very different method of behavior in the telophasic, interphasic, and prophasic stages.

Since the apparent structure and size of the chromosome of *Digitalis ferruginea* are similar to those of *Azolla caroliniana, Salvinia auriculata,* and *S. natans,* I attempted to determine whether or not there was a similarity in the chromosome behavior of *Digitalis ferruginea* and the two genera of water ferns mentioned, *Azolla* and *Salvinia.* It must be noted that de Litardiere's (1921) study was made of somatic mitosis, while this study has been concerned solely with the meiotic divisions.

During the months of June, July, and August of the year 1931, flower buds of *Digitalis ferruginea* were gathered. In order to be certain that material in the critical stages was secured, Belling's iron-aceto-carmine smear method was used to identify the various maturation stages. One anther from each bud was used to check the stages; the three remaining anthers in each bud, two usually older or younger than the anther used, were then

50 493

placed in Licent's [2] fixing solution. The killed and fixed anthers were embedded in paraffin by the usual method. The sections were cut from four to ten microns in thickness and stained in Heidenhain's iron-alum haematoxylin.

OBSERVATIONS

Size, shape, and number of chromosomes. From measurements of a representative group of chromosomes of *Digitalis ferruginea,* I found their length to vary from 0.4 to 1.6 microns, these measurements definitely placing *Digitalis ferruginea* in the class of plants having small chromosomes. These measurements were taken from chromosomes of a nucleus in the microsporocyte during the resting condition. Measurement of the chromosomes in the metaphase face view was impossible, due to the fact that the greater part of the chromosome is situated in the equatorial plane and in the face view only a portion of one end can be seen. It is possible to measure the chromosomes of the metaphase polar view, the length varying from 0.8 micron to 2.1 microns; this elongation is due to the activity during the preceding prophase.

During the prophase the chromosomes lengthen, losing their ovoid shape and assuming the shape of little rods more or less curved. They retain this increased length during the metaphase and anaphase, beginning to shorten during the telophase until the ovoid shape is regained and the length corresponds to that indicated above during the resting condition. Irregularity in the thickness of the various portions of the chromosome made it impossible to secure measurements of their width.

The various species of *Digitalis* have been made the subject of considerable investigation with regard to the number of chromosomes in the haploid and diploid generations. It is apparent from my observations and from these studies that the chromosomes are very numerous. Gaiser's (1930) compilation shows the haploid number in *Digitalis* species to vary from 24 to 48 chromosomes, the diploid number ranging from 48 to 96 chromosomes. Warren's low chromosome counts quoted in Gaiser (1930) seem inconsistent unless he was possibly dealing with a haploid individual.

During my investigation on *Digitalis ferruginea* I was able to count at least 24 bivalent chromosomes arranged in the equatorial plate, polar view (fig. 5).

Premeiotic interphase. In this phase the nucleolus attains its maximum size and the chromosomes retain their size, shape, and stainability. However, there appears a tendency for the chromosomes to clump together in aggregates or angular masses (fig. 1). At this stage anastomoses are plainly visible connecting the chromosomes which are dispersed throughout the entire nuclear mass. The anastomoses stain rather lightly in this stage as compared with the degree of color intensity they show in the prophase.

[2] Other fixing solutions were tried; none, however, proved to be superior to Licent's.
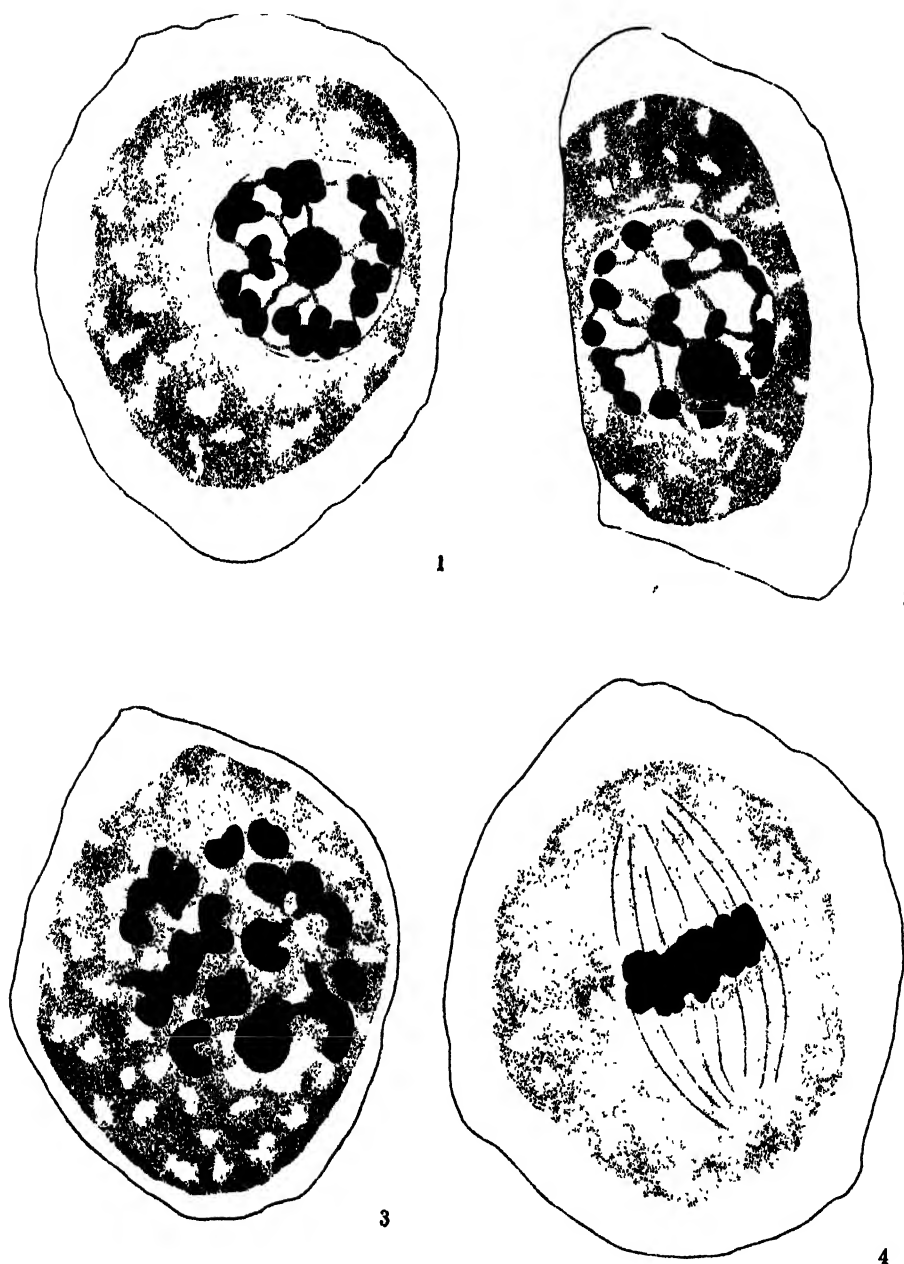
Fig. 1-4. *Digitalis ferruginea.* Fig. 1. Nucleus in premeiotic interphase, showing angular masses of chromosomes connected by anastomoses. Fig. 2. Nucleus in early meiotic prophase I, showing chromosome aggregates breaking up. Fig. 3. Nucleus in late prophase, showing rod-shaped chromosomes migrating toward one side of nucleolus, and disappearing anastomoses. Fig. 4. Metaphase, face view.

These anastomoses do not appear to be arranged in a spiral manner but rather as connecting strands extending from one chromosome or group of chromosomes to another individual or aggregate. Some of the anastomoses extend through the central area to the nucleolus (fig. 1).

Prophase. The first sign of anachromatic or prophasic activity in the primary microsporocyte is the dissociation of the chromatic aggregates, the chromosomes having a tendency to migrate toward one side of the nucleolus (fig. 3). During the prophase the anastomoses gradually disappear until at the end of this phase there remain but slight traces of the former connecting strands (fig. 3).

Simultaneously with the disappearance of the anastomoses, the chromosomes elongate and become more slender until they no longer appear as round or ovoid bodies but rather as relatively short rods resembling bacteria of the bacillus type (fig. 3). At this stage the bivalent chromosomes are scattered throughout the entire nucleus.

The nucleolus disintegrates slowly and completely until at the end of the anachromatic process nothing visible remains of it.

Since the chromosomes enter the prophase as distinct, visible individuals, the only observable changes during the prophase are (1) the change of shape of these bodies which become elongated and more slender, (2) the disappearance of the connecting anastomoses, and (3) the final disappearance of the nucleolus.

Metaphase. During this stage the chromosomes behave like ordinary large chromosomes in any, mitotic or meiotic division, coming to lie at the equator of the spindle in the center of the cell. Constrictions in chromosomes were not observed, nor could the exact method of attachment of the very distinct spindle fibres be determined (fig. 4). In a polar view of the spindle the chromosomes seem to be rather irregularly arranged (fig. 5), some taking up a peripheral position, others arranging themselves in a scattered group in the center of the plate. The chromosomes in this stage appear as short, curved rods, exactly like those of the late prophase.

Anaphase. In this stage the members of the pairs of homologous chromosomes begin to separate, gradually moving away from the equatorial plane to the opposite poles of the spindle. This migration continues until all the chromosomes of the group come into contact with one another, forming a compact mass (fig. 6). Sharp (1926) states that although it is probable that poor fixation may serve to accentuate this compactness in certain cases, there can nevertheless remain no doubt that, in general, a very close grouping of the chromosomes occurs naturally. De Litardiere's (1923) sole purpose for the paper " Fixation " was to prove that the close polar grouping is a normal, actual phenomenon, and not due to fixation fluids.

Telophase. In the massed group of chromosomes characterizing the end of the anaphase (fig. 6), individual chromosomes begin to appear. It seems as if the whole mass breaks apart and some repellent force pushes the members
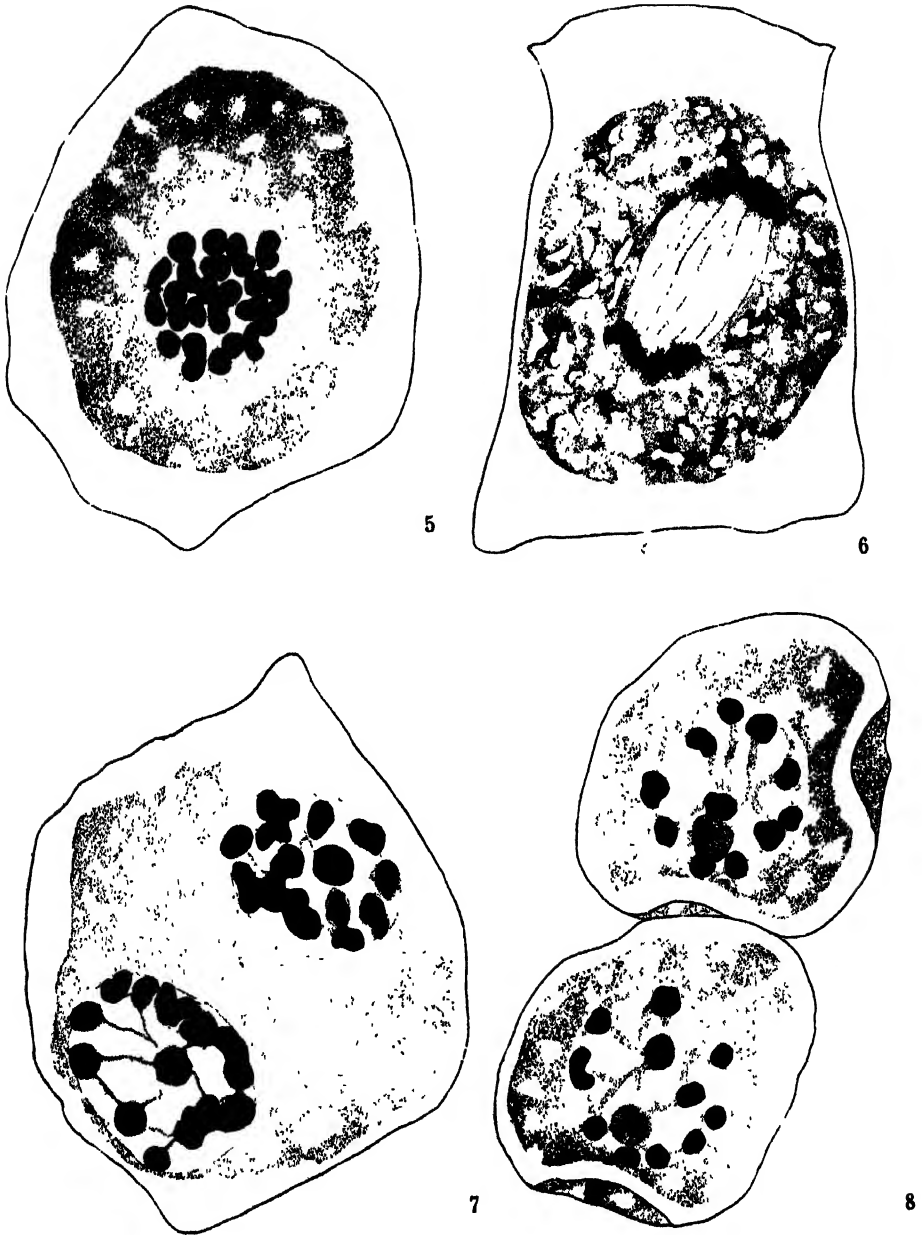
Fig. 5–8. *Digitalis ferruginea*. Fig. 5. Metaphase, polar view. Fig. 6. Anaphase, showing polar massing. Fig. 7. Nuclei in telophase, showing round chromosomes connected with nucleolus by anastomoses. Fig. 8. Young secondary microsporocytes, showing individual chromosomes located around periphery of nucleus.

away from one another until each short, ovoid chromosome is located near the periphery of the nucleus (fig. 7). During this change in position of the chromosomes the single nucleolus makes its appearance. (In none of the nuclei was there observed at any time more than one nucleolus.) The nucleolus becomes a fairly large body, its diameter approximating one sixth the diameter of the entire nucleus. It usually occupies a position near the center of the nucleus (fig. 7).

Fine anastomoses, staining lightly, appear at this stage, apparently connecting the chromosomes with each other, and extending through the central region of the nucleus to the nucleolus (fig. 7).

There is no apparent alteration in the internal structure of the chromosomes in the telophase, the chromosomes appearing to be of equal density throughout their length. The only changes in the chromosome proper are in size and shape; from rod-like bodies of the metaphase and anaphase, the chromosomes by shortening and thickening become round or ovoid bodies, a form they retain through interkinesis to the next prophase (fig. 7).

As a result of this behavior the catachromatic phenomenon or telophase transformation is greatly reduced. The complete process may be summed up into two steps: (1) A simple separation of the chromosomes accompanied by a change in size and shape. (2) The formation of fine anastomoses connecting the chromosomes.

With my technique, these anastomoses take a light lavender stain in contrast to the heavily stained chromosomes.

With the daughter nuclei completely formed, the daughter cells enter a period of rest (fig. 8); then the second of the meiotic divisions takes place, ending in the formation of the microspore tetrads. The short, ovoid chromosomes arranged around the periphery of the nucleus showed very plainly in the nuclei of the pollen grains, anastomoses being present here as in the preceding interphase stage.

DISCUSSION

Since the proposal of the chromosome theory of heredity, the importance of the question of the individuality of the chromosome has been more than ever appreciated. All evidence bearing on this question has been welcomed. While the genetical evidence that the chromosomes maintain their individuality from one cell generation to another has been abundant, there has been available little visual evidence supporting the hypothesis.

Sharp (1926, p. 171) states that in tissue with rapidly dividing cells the telophasic transformation of the chromosomes and their anastomoses to form the reticulum often do not proceed far enough during the interphase to obliterate the boundaries between the chromosomes, which separate again in the ensuing prophase without having lost their visible identity. In such nuclei, there can be little doubt that the individuality of the chromosomes is preserved.

· Further evidence bearing on the continuity of individual chromosomes

is found in the reports of investigations on plants having very small chromosomes. The investigations of de Litardiere (1921) on the chromosome behavior in *Azolla* and *Salvinia* show that these small chromosomes maintain their continuity as individual bodies through all phases of mitotic divisions.

In the present investigation it has been found that *Digitalis ferruginea*, having chromosomes with length measurements of from 0.4 micron to 1.6 microns, may be classed with plants having small chromosomes. It has also been demonstrated that in *Digitalis ferruginea* the chromosomes retain their visible identity throughout the two meiotic divisions in the microsporocytes.

From my observations it is quite evident that the telophasic transformation ceases at the stage where individual chromosomes reappear from the polar attachment connected by anastomoses (fig. 7) and become arranged at the periphery of the nucleus. This observation is in accord with the observations made on other species having small chromosomes—*Azolla caroliniana* by de Litardiere (1921), *Salvinia natans* by Yasui (1911) and de Litardiere (1921), squash species by Eichhorn (1930), and *Genetiana procera* by Denniston (1913).

During the premeiotic interphase (fig. 1) I was unable to find any structural transformation of the chromosomes in either diameter or general appearance, the only noticeable change being a grouping together of some of the chromosomes (fig. 1). This change in relative position of the chromosomes was also observed by Denniston (1913) and Yasui (1911). De Litardiere (1921), on the other hand, found no such grouping of the chromosomes, but rather a scattered arrangement very similar to the arrangement of the chromosomes in the telophase stage. In the reports of de Litardiere (1921) and Yasui (1911), however, I found no mention of a structural change during this phase. None of these investigators report internal changes in the chromosomes in either the telophase or the interphase. I was unable to see any such changes in the chromosomes of *Digitalis ferruginea* during any of the division phases.

Associated with this matter of small chromosomes whose identity is retained throughout the successive phases of division is the question as to the formation of a continuous spireme in such nuclei. Among investigators reporting on the behavior of nuclei with small chromosomes there is disagreement as to the presence of such a spireme. The present investigation furnishes evidence bearing on this topic.

The prophase is the stage in division in which there is the most disagreement in interpretation. De Litardiere (1921) interprets the prophase figures as showing not a spireme which finally breaks up into chromosomes, but individual chromosomes with anastomoses between them. Eichhorn (1930) interprets the prophase as showing the activity characteristic of prochromosomes. Yasui (1911) sees in the prophase figures of *Salvinia natans* a "delicate linin-reticulum," but individual chromosomes. In the early prophase of *Gentiana procera* Denniston (1913) finds a spireme which later breaks up into chromosomes.

In *Digitalis ferruginea* I had no difficulty in following the prophase from its beginning to the initiation of the metaphase. The first change I observed was a breaking up of the chromosome aggregates of the premeiotic interphase (fig. 2). At this time conspicuous strands, staining dark gray by my technique, could be seen extending throughout the whole interior of the nucleus (fig. 2).

I am not in favor of speaking of these strands as portions of a spireme, because of the presence of the clearly defined chromosomes (fig. 2). I am much more in favor of calling them anastomoses, even though they are apparently more pronounced in *Digitalis ferruginea* than they appear to be in de Litardiere's (1921) plates of *Azolla caroliniana*.

As the prophase activity proceeds, the chromosomes tend to migrate toward one side of the nucleolus, at the same time elongating, becoming slender, and assuming the shape of short curved rods. The anastomoses disappear rapidly, and the two members of each of several pairs of homologous chromosomes can be seen (fig. 3).

These observations of the later prophase seem to be in agreement with the observations made by de Litardiere (1921), Yasui (1911), and Denniston (1913).

Two theories regarding the behavior of the anastomoses during the late prophase have been suggested by de Litardiere (1921): (1) that these anastomoses gradually lose their stainability, or (2) that the anastomoses retract. Which of the two theories best suits the case in *Digitalis ferruginea*, I am unable to say.

I have not found in any of the reports of work done in *Azolla caroliniana, Salvinia natans, Gentiana procera,* and squash species any mention of any unusual behavior of the chromosomes during either the metaphase or the anaphase. The special behavior of small chromosomes seems to be confined to the telophasic and prophasic activity, and consists in each case in a considerably shortened and simplified process as compared with the more complicated processes found in those plant species characterized by long or slender chromosomes.

SUMMARY

In my investigation of the meiotic processes in the microsporocytes of *Digitalis ferruginea*, I found the following features:

1. During the premeiotic interphase the individual chromosomes, connected by fine anastomoses, remain distinct at all times. They have a tendency to group together in various positions throughout the nuclear mass, usually near the periphery of the nucleus.

2. These chromosomes pass through the meiotic divisions to the pollen grain nuclei as complete individuals without losing their visible identity at any stage.

3. Since the chromosomes enter the prophase as distinct individuals, the

prophasic activity is a simple process in *Digitalis ferruginea* and is in accord with the findings of de Litardiere (1921), who worked with *Azolla* and *Salvinia*. It consists of a change in size and shape of the individual chromosomes accompanied by a disappearance of the anastomoses, and does not resemble the behavior characteristic of prochromosomes.

4. The metaphase and anaphase are apparently much the same as described for these phases in such species as *Vicia faba, Lilium regalis, Tradescantia virginiana,* and other species of angiosperms with large chromosomes.

5. The telophase consists of a simple separation and shortening of the individual chromosomes, accompanied by the formation of fine anastomoses connecting the chromosomes with each other.

6. While pronounced or distinct anastomoses connecting the chromosomes are found in the early prophase, no structure which may properly be regarded as a continuous spireme has been observed.

In conclusion, I wish to express my sincere gratitude to Dr. J. Ben Hill, Mrs. J. Ben Hill, Dr. H. W. Thurston, Jr., and Dr. J. W. Sinden for their assistance in this problem, at the same time acknowledging my indebtedness to Dr. L. W. Sharp, who gave final criticism of the manuscript.

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A COMPARISON OF OSMOTIC CONCENTRATIONS OF SUPPLYING AND RECEIVING TISSUES AND ITS BEARING ON THE MÜNCH HYPOTHESIS OF THE TRANSLOCATION MECHANISM

OTIS F. CURTIS AND H. T. SCOFIELD

Münch (1930) has recently proposed a mechanism which he thinks accounts for the movement of solutes through phloem tissue. In brief, the Münch hypothesis proposes that there is a mass flow of solution from the supplying cells to the receiving cells, that the flow from cell to cell in the phloem passes through the pores of the sieve plates, while for the less specialized supplying or receiving cells the flow is through the more minute plasmodesma. The hypothesis demands that the supplying cells have a higher turgor pressure than the receiving cells. This would mean that, except under rather special conditions, the supplying cells must have a higher osmotic concentration than the receiving cells. There are several points about the hypothesis that are in its favor and make it rather intriguing, but there are also several grave weaknesses involved. No attempt will be made in this paper to summarize these various points, because a rather extensive criticism will be included in a book by the senior author that is shortly to appear and which deals with many of these and other interesting problems relating to solute translocation in plants.

According to the Münch hypothesis, the supplying cells must have a higher turgor pressure than the receiving cells so that there will be a pressure gradient forcing the solution along the channels leading to the receiving cell. General observations, however, give one the definite impression that receiving tissues are often more turgid than the supplying tissues. For example, the appearance of turgid sprouts developing from increasingly flaccid potato tubers is frequently to be seen, as is also the development of turgid shoots from storage roots, as of carrots, beets and turnips, and from other fleshy storage organs, such as bulbs and corms. The cotyledons of developing seedlings also appear less turgid than the receiving tissues.

Quantitative data comparing osmotic concentrations of receiving and supplying tissues are not extensive. The senior author (1920) in experiments incident to translocation studies obtained data indicating an osmotic gradient but with the higher concentrations near the growing tissues which were at the same time the receiving tissues. Fernald (1925) in testing this point presents rather extensive data demonstrating such a reverse gradient leading from

potato sprouts to the mother tubers from which they were receiving their supplies. A similar strong reverse gradient was also apparent in developing shoots of *Ligustrum* and *Philadelphus*. In these the terminal growing parts of the shoots consistently showed a higher osmotic concentration than the lower parts of the stem or the leaves from which the solutes were presumably coming. It is especially interesting that terminal growth ceased at about the time the leaves attained an osmotic concentration higher than the apex of the shoot.

It would seem that further data on pressure differences between supplying and receiving cells would enable one more critically to test the Münch hypothesis. We know of no method whereby turgor pressures can be directly measured. Indirect methods might be used, but there are serious difficulties and sources of error in using the indirect methods and especially in applying them to the type of material available. The data here reported, therefore, are restricted to measurements of the osmotic concentrations of supplying and receiving tissues.

It is true that there need be no predictable relation between osmotic concentration and turgor pressure; but on the other hand, if two tissues are organically connected so that there is ready communication, especially through the water-conducting system, and if both are placed in the dark at a high humidity so that neither tissue is subjected to conditions favoring rapid loss of water, and both are at the same temperature, then there is likelihood that turgor pressures of different tissues will be approximately proportional to their respective osmotic concentrations. Absence of light also precludes the possibility of complications due to localized photosynthesis. The growth of tissues developing from storage organs under these conditions is undoubtedly dependent upon the transport of organic materials from the storage organ to the growing parts. An effective mechanism is therefore functioning, and measurements of the osmotic concentrations in the supplying and receiving cells should indicate whether the mechanism is or is not dependent upon an osmotic gradient.

Osmotic concentrations of receiving and supplying tissues were calculated from measurements of the freezing point depressions of the respective tissues. In order to avoid difficulties and errors attendant upon extraction of sap, most of the measurements were determined by the thermocouple method, in which thermocouples, made by fusing number 36 wires of copper and " constantan," were used. These were inserted directly into the tissue to be tested, and the freezing point of the living tissue was directly measured without previous killing or other treatment. In most cases comparisons were also made with the freezing point of extracted sap. The apparatus used consisted of a Leeds and Northrup Type K-2 potentiometer, a Leeds and Northrup Type R galvanometer, sensitivity 0.5 microvolt, a standard cell, and two ordinary 1.5-volt dry cells. A thermos bottle kept full of ice and distilled water served as the cold junction. A graph of standardized tempera-

TABLE I. *Depressions of the freezing points, in degrees centigrade, of receiving and supplying tissues of onion and bean, and maximum osmotic pressures calculated from these*

															Av.	Odds	Osmotic pressure (atm.)
Onion tissues																	
Growing tissue...1.34	1.39	1.01	1.06	1.17	1.08	1.15	1.58	1.44	1.75	0.57	0.85	—	—		1.179	—	14.19
Storage tissue....1.00	1.04	0.88	0.85	0.96	0.79	0.94	1.38	1.12	1.43	0.44	0.43	—	—		0.918	—	11.06
Difference.......0.34	0.35	0.13	0.21	0.21	0.29	0.21	0.20	0.32	0.32	0.13	0.42	—	—		0.262	9999	3.13
Onion expressed sap																	
Growing tissue...0.73	0.70	0.75	—	—	—	—	—	—	—	—	—	—	—		0.73	—	8.79
Storage tissue....0.20	0.19	0.12	—	—	—	—	—	—	—	—	—	—	—		0.17	—	2.05
Difference.......0.53	0.51	0.63	—	—	—	—	—	—	—	—	—	—	—		0.56	458	6.74
Etiolated bean seedlings																	
Growing shoot...1.71	1.01	1.38	1.40	1.48	0.50	1.37	1.29	1.37	1.40	2.43	1.91	1.77	1.79		1.49	—	17.92
Cotyledon......0.68	0.88	1.05	1.22	1.06	0.57	1.32	1.13	1.27	1.52	1.92	1.47	1.77	1.62		1.25	—	15.04
Difference......1.03	0.13	0.33	0.18	0.42	-0.07	0.05	0.16	0.10	-0.12	0.51	0.44	0.00	0.12		0.238	207	2.88
Etiolated bean seedlings expressed sap																	
Growing shoot...0.86	0.84	0.64	0.67	—	—	—	—	—	—	—	—	—	—		0.75	—	9.03
Cotyledon......0.76	0.77	0.49	0.54	—	—	—	—	—	—	—	—	—	—		0.64	—	7.71
Difference......0.10	0.07	0.15	0.13	—	—	—	—	—	—	—	—	—	—		0.11	241	1.32

ture readings was made with the aid of a standard thermometer graduated to tenths of a degree centigrade.

In table 1 are presented data from onions (*Allium cepa* L.) and bean seedlings (*Phaseolus vulgaris* L. var. red kidney). In each case the plants were growing in the dark. With the onions, one thermocouple was inserted into one of the fleshy scales, which was acting as a supplying tissue, and the other was inserted into the basal part of the young developing leaves. With the beans, one thermocouple was inserted into the cotyledon and the other entered the plumular hook, penetrating towards the meristem. Although the thermocouples inserted into the receiving tissues were not in every case inserted into the meristem proper, they were undoubtedly in regions that were growing rapidly and were therefore receiving solutes from the storage organs.

From the table it is clear that the receiving tissues consistently have a higher concentration than the supplying, storage tissues. There were only two of the 65 determinations that indicated the contrary, and these two were in the bean seedlings in which measurements were more uncertain because of the small mass of tissue to be frozen. The odds, as calculated by Love's modification of Student's method (1924), show that even with the beans the differences are undoubtedly significant. These particular determinations show that, on the average, the receiving onion tissue had an osmotic concentration that could develop a pressure 3.13 atmospheres higher than the supplying tissue. The extracted saps showed a difference of 6.74 atmospheres in the same direction. The receiving tissue in the bean seedling had a concentration capable of developing an osmotic pressure 2.86 atmospheres higher than the supplying tissue, while the extracted saps showed a difference equivalent to 1.32 atmospheres.

It is noticeable that the concentrations of the expressed saps are distinctly lower than those of the living tissues. This is in agreement with the findings of Carrick (1930). The killing in each case has evidently altered the composition in the same direction but to different degrees. A few preliminary tests by the plasmolytic method with onion point to the direct freezing as more nearly correct than the sap-extraction method. Such data giving the

TABLE 2.   *Osmotic concentrations, at incipient plasmolysis, of storage and receiving tissues of onion as determined by the plasmolytic method with volume molecular solutions of sucrose, and the freezing points of the sucrose solutions used.*

							Av.	Osmotic pressure (atm.)
Growing tissue	Mol. sucrose.......	0.55	0.62	0.60	0.58	0.50	0.57	13.24
	Freezing point of sucrose.......	1.03	1.30	1.15	1.13	0.97	1.10*	
Storage tissue	Mol. sucrose......	0.11	0.22	0.22	0.24	0.17	0.19	4.10
	Freezing point of sucrose.......	0.18	0.39	0.39	0.44	0.31	0.34*	
Difference.....	Mol. sucrose.......	0.44	0.40	0.38	0.34	0.33	0.378	9.14

* Direct freezing points of sugar solutions made up to average strength.

TABLE 3. *Depressions of freezing points, in degrees centigrade, of receiving and supplying tissues of Bryophyllum, squash seedlings, potato and Byrnesia and osmotic pressures calculated from these*

															Av.	Odds	Osmotic pressure (atm.)
Bryophyllum mother leaf and plantlet																	
Growing plantlet....0.75	0.73	1.05	1.57	1.36	1.05	1.13	1.07	0.90	0.92	1.10	0.91	0.70	0.86	1.02	1.008		12.14
Mother leaf....0.57	0.53	0.89	1.36	1.18	0.74	0.85	0.78	0.53	0.77	0.77	0.65	0.65	0.65	0.65	0.771	9999	9.29
Difference....0.18	0.20	0.16	0.21	0.18	0.31	0.28	0.29	0.37	0.15	0.33	0.26	0.05	0.21	0.37	0.237		2.85
Squash seedlings																	
Growing shoot..1.12	1.60	1.44	1.50	0.67	1.15	1.00	1.49								1.246		14.99
Cotyledon....1.02	1.00	1.32	1.29	0.60	1.07	0.70	1.43								1.054	87.5	12.69
Difference....0.10	0.60	0.12	0.21	0.07	0.08	0.30	0.06								0.193		2.30
Potato tissues																	
Tip of growing sprout......1.80	2.50	2.44	2.16	2.70	2.69										2.382		28.60
Mother tuber...1.70	1.96	1.34	1.75	1.94	2.38										1.845	138	22.18
Difference....0.10	0.54	1.10	0.41	0.76	0.31										0.537		6.42
Byrnesia plantlets																	
Plantlet......0.71	0.66	0.50													0.62		7.47
Mother leaves. 0.40	0.49	0.40													0.43	20.7	5.18
Difference....0.31	0.17	0.10													0.19		2.29

osmotic concentration at incipient plasmolysis, as determined with sucrose, are given in table 2.

In addition to the experiments with onion and bean, freezing point measurements of supplying and receiving tissues were made on mother leaves of *Bryophyllum pinnatum* Kurz. and the plantlet developing from them; cotyledons and growing shoots of squash seedlings (*Cucurbita maxima* Duchesne); potato tubers (*Solanum tuberosum* L.) and their developing sprouts; and leaves and plantlets of *Byrnesia* (*Echeveria*) *Weinbergii* Rose. With the *Bryophyllum*, one thermocouple was inserted into the mother leaf near the origin of the plantlet, and the other was inserted into a growing part of the young plantlet. In some cases this was inserted into a young leaf, and in others into the stem as near the growing point as possible. The data, presented in table 3, are in agreement with those in tables 1 and 2; that is, in each case the receiving tissue has a higher osmotic concentration than the storage tissues from which the solutes are moving. The osmotic concentration differences were such that under standard conditions the pressure differences would be 2.29, 2.30, 2.86, and 6.42 atmospheres for *Byrnesia*, squash, *Bryophyllum*, and potato, respectively.

It seems possible that other types of growing tissues may have lower concentrations than the supplying tissues. Hibbard and Harrington (1916), working with crushed tissues, observed that the roots of corn seedlings had lower osmotic concentrations than the tops. In order to determine the relative concentrations of growing root tips and supplying storage tissues, direct freezing point measurements were made of onions sprouted with their roots in water. Such measurements are given in table 4.

TABLE 4. *Freezing point depressions of root tips as compared with storage scales of onion*

	Outer	Middle	Middle	Inner	Av.	Osmotic pressure (atm.)
1. Storage scales......	0.37	0.62	0.62	0.70	0.58	6.98
Root tips......... 1.22	0.975	1.08 0.97	0.92 0.935	0.77 1.19	1.07	12.88
2. Storage scales......		0.87	0.88	0.87	0.87	10.48
Root tips.........0.99	1.02	0.89	1.10	1.12	1.02	12.28

From these data it is clear that the growing root tip also has a higher osmotic concentration than the supplying storage tissue. We cannot say whether the lack of agreement between these data and those of Hibbard and Harrington is due to the fact that in their case the roots were probably receiving food from photosynthetic organs while with the onions here reported it was coming from a storage tissue, or whether it was due to their including the entire root, growing and non-growing parts alike while the measurements here presented were restricted to actively growing tissues.

It is worth noting that these onion roots were growing in water while

the supplying tissues received their water through these same roots. It seems certain, therefore, that the root tips, with a higher concentration and standing directly in water, must have a turgor pressure in excess of that of the storage scales from which they were receiving solutes.

Since the outer scales of the onion seem to become emptied of their reserves before the inner scales, a few measurements were made to determine if an osmotic gradient existed between the outer and inner scales. Determinations were made with two groups, one sprouted in tap water standing in darkness in the laboratory, the other sprouted in a paper bag in a refrigerator at about 5 to 7°C. The data are presented in table 5.

TABLE 5.   *Freezing point depressions in different layers of sprouting onion bulbs*

	Bulb no.	Growing region	Storage scales numbered from the inside toward the outside				
			1	2	3	4	5
Onions sprouted in water in darkness.	1	—	0.87	0.43	—	—	—
	2	0.89	0.74	0.60	0.23	0.20	0.18
	3	—	1.19	0.62	—	—	—
	4	—	0.89	0.67	0.49	—	—
	5	1.77	1.49	1.28	0.97	—	—
	6	2.57	1.71	1.54	1.13	—	—
	7	1.98	1.37	1.12	0.88	0.85	—
	8	—	1.44	1.16	0.77	—	—
	9	1.64	1.20	0.64	—	—	—
	10	0.94	˙0.71	0.23	0.23	—	—
Onions sprouted in refrigerator.	1	—	1.58	1.26	1.15	—	—
	2	—	1.34	0.98	1.13	—	—
	3	—	1.24	1.26	1.04	0.59	—
	4	—	1.38	1.40	1.31	0.97	0.58
	5	—	1.06	1.34	—	—	—

From the table it is evident that, with onions sprouted with their roots in water, there is a clear-cut osmotic gradient leading from the young, growing region to the outer scales, the reverse of the requirements of the Münch hypothesis. With those sprouted without water, the gradient from one layer to another is not so regular. The concentrations of the growing regions were not determined for this series, but previous lots sprouted under similar conditions showed that this region consistently has a higher concentration as shown in table 1.

All of the data here presented demonstrate higher osmotic concentrations in the receiving tissues than in the supplying tissues. That this is always the case has not been demonstrated. It is possible that in some cases receiving tissues rather consistently may have lower osmotic concentrations. This may be the case with roots, although our data with onion root tips seem to oppose such a gradient. Chandler (1914) observed that growing fruits commonly had lower concentrations than the supplying leaves. Possibly tissues receiving solutes from leaves carrying on photosynthesis have lower concentrations than the supplying tissues. Fernald (1925), however, found

the shoot growing regions to have higher concentrations than the leaves and that elongation ceased when the leaf concentration exceeded that of the growing shoot. Whether storage tissues have or have not higher concentrations than the tissues supplying the materials remains to be tested. Although there are instances of this sort where translocation may take place from a region of high concentration to one of low concentration, the many exceptions reported in the present paper clearly demonstrate that transport frequently takes place against an osmotic gradient. The frequent exceptions, therefore, seem to vitiate the Münch hypothesis.

There is a possible weakness in the data here presented which deserves further comment. As previously mentioned, there need be no direct relation between osmotic concentration and turgor pressure. Demonstration of a higher concentration in the receiving cells, therefore, does not demonstrate a pressure gradient in the reverse direction. Ursprung and Blum (1924), in fact, report instances where the osmotic concentrations of outer cortical cells—for example, on two sides of a curving root of *Vicia faba*—were approximately equal but in which the turgor pressures on one side were estimated to be much higher than the other. Table 6 summarizes part of table 5 from the paper of Ursprung and Blum.

TABLE 6. *Summary of data of Ursprung and Blum*

Side	Osmotic value at incipient plasmolysis		Suction tension (atm.)	Turgor pressure (atm.)
	Mol. sucrose	Atm.		
Concave...........	0.4	11.1	0.45	7.45
Convex............	0.38	10.5	8.17	1.33
Concave...........	0.43	12.1	0.9	10.2
Convex............	0.45	12.6	7.0	3.0

From the data it seems that the growing cells on the convex side, which were also receiving cells, though probably not receiving solutes from the cells on the concave side, had very high suction tensions [1] and low turgor pressures when compared with the cells on the concave side. It might be claimed that the receiving cells, although they had higher osmotic concentrations than the supplying tissues, as shown in tables 1 and 2, because they were growing rapidly probably had a high suction tension and low turgor pressure as indicated by the data of Ursprung and Blum. This would allow for a pressure gradient which would favor transport to them even though the osmotic gradient was in the reverse direction.

There are two distinct objections to this interpretation, however. In the first place, unless the gain in volume of the growing cells equalled or exceeded

[1] The term turgor deficit would seem to be distinctly preferable to suction tension, but the latter is here used because of its common use in the literature.

the volume of the solution received, it would be necessary that the receiving cells excrete water. According to the Münch hypothesis, the receiving cells lose water because their contents are under a turgor pressure in excess of that developed by the osmotic value of their contents. Solution is forced into the cells under such a pressure that water is excreted. They would therefore be at maximum pressure and have approximately zero suction tension. The data of Ursprung and Blum indicate that they are not at maximum turgor pressure, but have a high suction tension. This would point to no secretion of water, and the Münch hypothesis would require modification eliminating the necessity of water excretion.

In the second place, it is possible that the high suction tensions, and therefore low turgor pressures as measured by Ursprung and Blum, are not natural but result from the method of examination. It is likely that tissues as near together as the cortical cells on two sides of a root are in approximate equilibrium so far as the suction tension is concerned. If the two sides had the great difference in suction tension reported—that is, six to eight atmospheres —it would seem that the side with the high suction tension should quickly withdraw water from the neighboring tissues with low suction tension, unless a water-proof layer intervened—which is doubtful. When the tissues are cut, however, as was done in preparing them for observation, restraining pressures, due perhaps to the enclosing epidermis, will be released. Then any cells with easily extensible or elastic walls will stretch and show a high suction tension and low turgor pressure, while other cells, with less extensible walls, will change but slightly in their suction tension and turgor pressure.

An easily demonstrable, though perhaps somewhat exaggerated example of this can be seen when splitting a dandelion scape. Before splitting, the encasing epidermal cylinder with thick, non-elastic cuticle keeps the cells compressed. On splitting, these pressures are released, the inner, thin-walled cells undoubtedly change both in size and shape, and the released pressure must result in an increase of suction tension over that before cutting. Comparable releases of pressure may largely account for much of the great difference in suction tension and turgor pressure observed by Ursprung and Blum. This source of error seems to have been overlooked or underestimated in some of the recent attempts to measure normal suction tensions. The degree of error will of course vary with the extent to which mechanical tension or pressures are altered by cutting. It is true that Ursprung and Blum recognize the possibility of released tensions and pressures attendant upon cutting and, for example, they explain the greater absorption of water by the concave side of bending roots of *Vicia faba*, when tangential sections are made, as due to the cutting, but they assume that, when median sections are taken, the relative pressures of convex and concave sides are unaltered and the measurements of the suction tensions of cells in such pieces indicate normal conditions. This assumption seems unjustified, especially in view of the fact that, in tangential sections, the suction tension of the convex side is

nearly zero and that of the concave side is higher, as indicated by the failure of the convex side to absorb water and the relatively great absorption by the concave side, while median sections show the reverse condition. We do not disagree with the main thesis and valuable contribution of Ursprung and Blum, that growth and bending are associated with wall extension and not increased turgor of the growing cells. It merely seems doubtful that tissues so closely associated can, before cutting, have the very great differences in suction tension reported.

Data here presented, especially in table 4, clearly point to a lower and not a higher suction tension in receiving tissues. The receiving roots of these onions were submerged in water while the supplying storage tissues were in the air. Since the root tips had also a higher osmotic concentration, it is hardly conceivable that they could have had a lower turgor.

SUMMARY

The hypothesis proposed by Münch to explain the mechanism of solute transport demands a turgor pressure gradient leading from the supplying tissue to the receiving tissue. He suggests also thât the turgor gradient is chiefly dependent upon an osmotic gradient.

Osmotic concentrations of several types of supplying and receiving tissues have been determined by direct freezing point measurements of the tissues concerned, by measurements of the freezing points of extracted saps, and by plasmolytic measurements with sucrose solutions.

Such measurements were made with the following types of materials: Potato tubers and the sprouts developing from them, cotyledons of beans and squash and growing points of the dependent seedlings, parent leaves of *Bryophyllum* and *Sedum* and the plantlets developing from them, storage scales of onions and the young leaves as well as root tips drawing their supplies from them.

For each type of material tested, the osmotic gradient leads from the receiving tissue to the supplying tissue. Although this does not conclusively demonstrate a turgor gradient in the same direction, it does strongly indicate such a gradient. General observations of great flaccidity in storage organs which are supplying more turgid growing tissues support the evidence from quantitative determinations of osmotic concentration that turgor gradients frequently, if not consistently, lead from the receiving cells to the supplying cells.

This evidence clearly tends to refute the hypothesis of Münch, which demands a pressure gradient in the reverse direction—that is, from the supplying cells to the receiving cells.

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THE DEVELOPMENT OF SEEDS IN CERTAIN ERICALES

HERBERT F. COPELAND

The ovule of *Monotropa Hypopitys* is a classic subject of study. It seems first to have been described by Müller (1847). The development of seeds in this species was described in detail by Koch (1882). Stevens (1911) published the first accurate description of the development of seed in a species of Ericaceae, *Epigaea repens*. Samuelsson (1913) summarized previous work on the embryology of Ericales and published his own studies of the Scandinavian species. From Samuelsson's work it appears that the course of events is remarkably uniform throughout the order, and that some of the stages are characteristic enough to be diagnostic. A more recent summary is included in Schnarf's (1931) " Vergleichende Embryologie der Angiospermen."

OBSERVATIONS

I have examined the ovules of some Californian species included by Engler and Prantl in the Pyrolaceae—namely, *Sarcodes sanguinea* Torr., *Chimaphila Menziesii* Spreng., *Pyrola picta* Sm., *P. dentata* var. *integra* Gray, *P. aphylla* Sm., *Pterospora Andromeda* Nutt., and *Pleuricospora fimbriolata* Gray. Of these species, *Sarcodes sanguinea* had previously been studied in considerable detail by Oliver (1890); the others, so far as I can learn, have not been studied from an embryological point of view. I have not obtained a complete series of stages in any one species. All of the stages which I have seen fit nicely into the course of events as described by Samuelsson; my observations tend, therefore, to confirm the uniformity of the order, and seem worth publishing for that reason. At the same time, I have noticed differences in detail, which may offer hints as to classification.

I have to thank Mr. Horace J. Child for help with the technique; for the conscientious accuracy of the figures I have to thank the artist, Miss Helen Rearwin.

The ovules of many Ericales, including those which I have studied, are minute and numerous on the placentae. Each ovule primordium in growing out soon becomes inverted, so that the ovules are anatropous. The integument is single: Müller, in his pioneer work, remarked with apparent surprise that he was unable to distinguish a " secundine." In the species of *Pyrola* and *Chimaphila* which I have examined, the integument (except along the

raphe) consists always of just two layers of cells. In *Pterospora Andromeda* there are two layers at first, but in this species and in *Pleuricospora fimbriolata* an intermediate layer can be detected when the ovule is ready for fertilization. In *Sarcodes* the integument consists, at the spore-mother-cell stage, of about three layers of cells, and at fertilization of about five layers.

The nucellus is a single evanescent layer of cells enclosing the large archesporial cell.

There is no parietal tissue; the archesporial cell is itself the spore mother cell. Figure 1 shows the spore mother cell of *Pterospora*, enclosed in the young ovule. I have observed stages of reduction division only in *Sarcodes*. In this plant I have seen anaphases, apparently of the heterotypic division. I have not been able accurately to count the chromosomes; the number is about fifteen. I can confirm Oliver's observation that exactly two " cap cells " are produced.

The large functional megaspore undergoes divisions resulting in the development of an embryo sac of the " normal " type, with an egg, two synergids, an endosperm mother cell,[1] and three antipodal cells. During the development of the embryo sac, and well before fertilization, the characteristic wing on the seed of *Pterospora* begins to form. It consists of two layers of cells, and is formed by the proliferation of the epidermal layer at the antipodal end of the ovule. Figure 2 represents the origin of this wing, the embryo sac being in a 4-nucleate stage. At this early stage the nucellus has been absorbed except for a fragment at the micropylar end; in all Ericales, as appears from the published accounts and from my own observations, it disappears before the embryo sac is mature.

Figure 3 represents the ovule of *Pyrola dentata* var. *integra*, including a fully developed embryo sac. Only one polar nucleus is shown in the figure; I have seen two in other sections of the same species, also in *P. picta* and *Chimaphila Menziesii;* in *Pterospora* I have seen a single large nucleus evidently formed by the union of the polar nuclei. I have not observed fertilization.

After fertilization, the endosperm mother nucleus divides and each of the daughter nuclei divides. The four resulting nuclei are arranged in a row on the long axis of the embryo sac. Cell divisions follow immediately upon the nuclear divisions; the result is a young endosperm consisting of a row of four cells. This stage is characteristic of all Ericales; it was first figured in Müller's early work on *Monotropa*. Figure 4 shows the four-celled endosperm of *Sarcodes;* I am able also to report its occurrence in *Pleuricospora*. According to Samuelsson, the endosperm as found in the seed is developed entirely from the two intermediate cells of the four-celled structure; in Ericaceae, the terminal cells give rise to " haustoria," while in

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[1] By this term is meant the large cell occupying the space between the antipodal cells and the egg apparatus. Text-books in general seem to avoid applying to it any definite name.
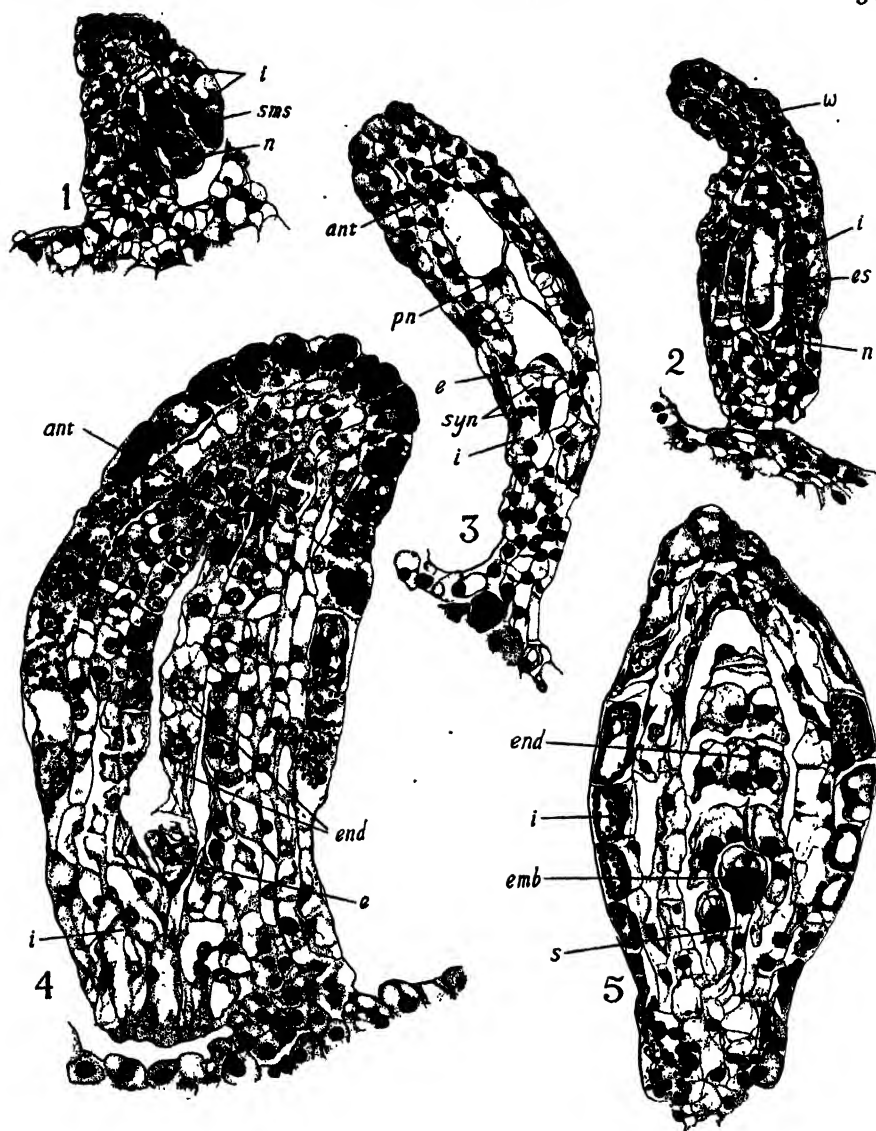
Fig. 1-5. All figures are magnified 280 diameters. The slides from which they were made are cited by number; these slides are deposited with the Department of Botany of the University of California. Explanation of abbreviations: *i*, integument; *n*, nucellus; *sms*, spore mother cell; *w*, wing; *es*, embryo sac; *syn*, synergids; *e*, egg; *pn*, polar nucleus; *ant*, antipodal cells; *end*, endosperm; *s*, suspensor; *emb*, embryo.

Fig. 1. Ovule of *Pterospora Andromeda*, showing the megaspore mother cell. Slide *45-4*.—Fig. 2. Older ovule of *Pterospora Andromeda*; the embryo sac 4-nucleate, the nucellus absorbed except for a fragment at the micropylar end, the wing beginning to develop. Slide *51-11*.—Fig. 3. Ovule of *Pyrola dentata* var. *integra*, including a fully developed embryo sac. Slide *33-12*.—Fig. 4. Developing seed of *Sarcodes sanguinea* showing the 4-celled endosperm. Slide *11-8*.—Fig. 5. Developing seed of *Pleuricospora fimbriolata* showing a many-celled endosperm and the suspensor bearing a young embryo. Slide *58-10*.

Pyrolaceae, whose ovules present very little tissue for haustoria to act upon, the terminal cells degenerate. I have seen stages between the four-celled endosperm and the mature seed only in *Pleuricospora,* and have been unable to determine the exact history.

The fertilized egg grows out into a slender suspensor which carries the one-celled pre-embryo into the interior of the endosperm, where it develops. The suspensor and the several-celled embryo of *Pleuricospora* are shown in figure 5.

At the time of fertilization the outermost layer of cells of the integument is distinguishable from the inner layer or layers by an accumulation of dark-staining material. As the seed develops, all of the integument except the outermost cell-layer is absorbed. This again is, according to Samuelsson, an ordinal character. The mature seed, which I have seen in *Sarcodes,* consists of a coat which is a single layer of dead cells with thick walls; an endosperm of many cells well packed with granular material and making up the bulk of the seed; and a small undifferentiated embryo imbedded in the endosperm near the micropylar end.

DISCUSSION

These observations permit certain remarks on the order Ericales and on the family Pyrolaceae.

The embryological characters of the order include the following: (1) A single integument; (2) a scant, ephemeral nucellus; (3) no parietal tissue; (4) an embryo sac of the " normal ' type; (5) a young endosperm consisting of four cells arranged in a row.

I have recently (Copeland, 1931) republished a hypothesis formerly put forward by Lindley: that the genus *Saurauia* represents essentially the ancestors of the Ericales. Evidence bearing on this hypothesis has in the past been drawn only from gross morphology. In Schnarf's work, I find embryological evidence of a confirmatory character: *Saurauia* is known to show all of the embryological characters listed above, except the fifth; it is known that the first division of the endosperm mother nucleus is followed by the formation of a transverse wall, but further stages are not reported.

As to the family Pyrolaceae, the genera currently included in it are well known to be diverse in gross characters: in the development of leaves and roots (depending on the different methods of nutrition in the different groups); in the corolla, which may be choripetalous or sympetalous; in the pattern of the anthers; and in the nature of the fruit, which may be a loculicidal capsule (in *Pyrola, Chimaphila,* and *Pterospora*), a capsule of peculiar type, opening by a circular cleft about the base of the style (in *Sarcodes*), or a berry (in *Pleuricospora*). My observations on the ovules reveal differences of similar weight. The ovule of *Pterospora* differs from that of *Pyrola* or *Chimaphila* in the peculiar wing, as well as in a somewhat better developed integument; while the integument of *Sarcodes* is comparatively massive.

It is my belief that the family Pyrolaceae is not a natural group; but it will require a long series of investigations to associate the various genera included in it with their nearer relatives.

SUMMARY

Stages in the development of ovules and seeds of *Sarcodes sanguinea* Torr., *Chimaphila Menziesii* Spreng., *Pyrola picta* Sm., *P. dentata* var. *integra* Gray, *P. aphylla* Sm., *Pterospora Andromeda* Nutt.. and *Pleuricospora fimbriolata* Gray have been observed. The course of development is found to be as previously described for the Ericales by Samuelsson and Schnarf, but differences between the genera, particularly in the development of the integument, are found to exist. Resemblance between the embryology of the Ericales and that of *Saurauia* is pointed out.

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A TAXONOMIC STUDY OF THE GENUS NAMA. II[1]

C. Leo Hitchcock

Sect. V. EUNAMA—CONTINUED

KEY TO THE ANNUAL SPECIES

Plants of Hawaiian Islands.....................................24. *N. sandwicense.*
Plants not of Hawaiian Islands.
  Small prostrate matted plants; corolla 3–5 mm. long; North American.
    Plants of Mexico; capsules 50–90 seeded, glabrous........29. *N. torynophyllum.*
    Plants of California; capsules 16–40 seeded, pubescent.
      Capsules 20–40 seeded; leaves rhombic-ovate to obovate, 6–13 mm. long.
                                   30. *N. pusillum.*
      Capsules 16–30 seeded; leaves spatulate, 10–30 mm. long.....31. *N. depressum.*
  Plants more erect, or if prostrate and matted, then either the corolla 6 mm. or more
    in length, or the plants from Mexico or Chile.
    Leaves, at least the cauline ones, sessile and markedly clasping, or strongly undulate.
      Filament-scales wide, usually with free tooth above the adnate base.
                                  19. *N. Schaffneri.*
      Filament-scales narrow, without free tooth above the adnate base.
        Densely villous; corolla 5–6 mm. long; plants of Chile.
                          20b. *N. undulatum* var. *australis.*
        Scarcely or not at all villous; corolla 6–11 mm. long; plants not native of
           Chile.
          Corollas 8–11 mm. long; leaves few; plants slender.
                        20a. *N. undulatum* var. *macranthum.*
         Corollas 6–9 mm. long; leaves numerous; plants robust.
                           20. *N. undulatum.*
    Leaves sometimes sessile, but not markedly clasping or strongly undulate.
      Petioles decurrent on stem, the stem thus winged.
        Styles ca. 4 mm. long, connate at least 1/3 their length; flowers on filiform
          pedicels 1–4 cm. long............................16. *N. biflorum.*[2]
        Styles ca. 1.5–2 mm. long, usually free; flowers usually (not always) on
          shorter pedicels...............................18. *N. jamaicense.*
      Petioles not decurrent on stem, the stem not winged.
        Leaves linear-lanceolate, strigose........................22. *N. Stevensii.*
        Leaves various, but not linear-lanceolate.
          Plants prostrate, matted, villous-hirsute; leaves seldom over 4 mm. broad;
           seeds brown.
          Leaves rhombic-obovate, about 4 mm. broad.
                          28a. *N. demissum* var. *Covillei.*
         Leaves not rhombic-obovate, averaging less than 4 mm. broad.

[2] *N. biflorum* and *N. Havardii* were treated in part I of this study.

Calyx silky-pilose; leaves ca. 1 mm. broad; plants of Lower Cali-
fornia...................28c. *N. demissum* var. *linearis.*
Calyx not silky-pilose; leaves over 1 mm. broad; plants of n.
Lower California or United States.
Leaves averaging about 3 mm. broad; plants of Nevada and
Utah................................28. *N. demissum.*
Leaves averaging about 1.5 mm. broad; plants of Arizona,
California, and n. Lower California.
28b. *N. demissum* var. *deserti.*
Plants ascending or erect, or if prostrate, leaves over 5 mm. broad or
seeds yellow and herbage more or less hispid.
Styles connate to middle......................32. *N. Ehrenbergii.*
Styles not connate to middle.
Adnate portion of filaments longer than free portion.
17. *N. Havardii.*
Adnate portion of filaments not longer than free portion.
Scales of filaments wide, projecting above as tooth.
19. *N. Schaffneri.*
Scales of filaments narrow or lacking, not projecting above into
tooth.
Seeds with large pits; corolla 3-7 mm. long (sometimes
longer in no. 27a).
Leaves linear-elliptic to narrowly spatulate, 1-4 mm.
broad......................27. *N. dichotomum.*
Leaves oblong-elliptic to broadly spatulate, 2-15 mm.
broad.
Corolla 4-7 mm. long; styles 1.5-6 mm. long; plants
well-branched, very floriferous, 15-50 cm. tall.
27a. *N. dichotomum* var. *chasmogamum.*
Corolla 3-5 mm. long; styles 0.5-2 mm. long; plants
5-25 cm. tall, sparingly branched, not very
floriferous.
Calyx and leaves grayish hirsute.
27b. *N. dichotomum* var. *pueblense.*
Calyx and leaves not grayish-hirsute.
North American plants; sepals twice as broad
at apex as at base.
27c. *N. dichotomum* var. *latisepalum.*
South American plants; sepals scarcely twice
so broad at apex as at base.
27d. *N. dichotomum* var. *amplifolium.*
Seeds not large-pitted (condition not known in no. 26);
corolla 7-15 mm. long.
Pedicels 2-6 mm. long; seeds brown (not known in
no. 26).
Leaves 2-6 mm. broad...........25. *N. parvifolium.*
Leaves 6-12 mm. broad..........26. *N. prostratum.*
Pedicels 1-2 mm. long; seeds yellow when mature.
Adnate portion of filaments twice as broad as the
free portion; branches filiform; plants soft-
pubescent.
Leaves 3-8 mm. broad, gradually attenuate to
base......................23. *N. Coulteri*

Leaves 5–10 mm. broad, distinctly petioled.

    23a. *N. Coulteri* var. *Pringlei.*

Adnate portion of filaments not twice so broad as the free portion; branches more rigid; plants usually somewhat hispid.

Leaves strongly revolute and soft-pubescent; plants from near the lower portion of the Colorado River.

    21b. *N. hispidum* var. *revolutum.*

Leaves frequently revolute, but usually rather hispid.

Plants strict, erect, branching from about middle.................21. *N. hispidum.*

Plants profusely branched from base, ascending to nearly prostrate.

Sepals linear-spatulate; corolla 12–15 mm. long...21d. *N. hispidum* var. *sonorae.*

Sepals linear-lanceolate; corolla 8–14 mm. long.

Leaves glandular beneath; hispid pubescence of leaves confined to upper surface, margins, and midrib of lower surface.

    21c. *N. hispidum* var. *Mentzelii.*

Leaves rather uniformly hispid, not markedly glandular.

    21a. *N. hispidum* var. *spathulatum.*

18. N. JAMAICENSE Linn. Syst. Nat. ed. 10, **2**: 950. 1759; Choisy, DC. Prodr. 10: 182. 1846; Gray in Hemsl. Biol. Cent.-Am. Bot. **2**: 365. 1882; Brand, l. c. 155. *N. jamaicense* var. *gracile* Brand, l. c. 156 (merely an ecological variant). *N. reclinata villosa* P. Browne, Civ. & Nat. Hist. Jam. 185. t. 18, fig. 2. 1752, acc. Brand. *Hydrolea jamaicense* Roenschel, Nom. ed. 3, 76. 1797. *Hydrolea decurrens* Moc. ex Choisy, l. c. 181. *Conanthus jamaicensis* (L.) Heller, Cat. N. Am. Pl. 6. 1898. *Marilaunidium jamaicense* (L.) Kuntze, Rev. Gen. Pl. **2**: 434. 1891. Pl. 27, fig. 19.

A slender, prostrate or ascending, strigose-hirsute annual, the branches rather simple, 10–50 cm. long; leaves variable, from rotund or broadly ovate to spatulate or broadly obovate-spatulate, 1.5–8 cm. long, 0.5–3.5 cm. broad, narrowed to distinct petioles ¼ as long to as long as the blades, petioles decurrent, the stems thus winged; flowers rather few, borne singly or in 2's or 3's along the stem, from nearly sessile to pedicellate, the peduncles and pedicels stout or slender, as much as 1.5 (5) cm. long; calyx-lobes linear or narrowly spatulate, ca. 5 mm. long in flower, increasing in length in fruit and usually adhering rather tightly to the ovary, but the ovary not inferior; corolla almost tubular, 6–7 mm. long; stamens unequally inserted 1–2 mm. from base of corolla-tube, terete above, but flattened and greatly expanded about 1 mm. above point of insertion, the adnate portion expanded and with free margins; styles 1.5–2 mm. long, usually distinct, but sometimes united as much as half their length; ovary elongate, usually with hardened, adherent calyx-lobes, 50–70-seeded; seeds light brown, ca. 0.5 mm. long, similar to those of *N. biflorum,* the alveolae perhaps somewhat smaller.

Representative material—UNITED STATES: Florida: Key West, *Blodgett* (G); Texas: without locality, *Lindheimer 476* (C, G, M); Austin, *Hall 677* (M, P); San Antonio, *Clemens and Clemens 482* (CA, M, P). MEXICO: without locality, *Berlandier 2049 = 639* (C, M); Tamaulipas—Matamoras, *Berlandier 2298* (G); Sinaloa-Culiacan, Sept. 19, 1904, *T. S. Brandegee* (C, G); vic. of San Blas, *Rose, Standley and Russell 13199* (US); San Luis Potosi—Minas de San Rafael, *Purpus 5346* (C). YUCATAN: San Anselmo, *Gaumer 1602* (C, M, P, US), *1657* (C, CA, G, M, US), and *1655* (C, G, US); without locality, *Valdez 15* (G, M, US), *Gaumer 326* (G, M, US). GUATEMALA: Coban, Dept. Alta Vera Paz, *von Turckheim 1266* (G, US) and *8* (US, with connate styles). EL SALVADOR: vic. of San Miguel, San Miguel, *Standley 21144* (G, US). HONDURAS: between Llano de la Puerta and El Salto, *Pittier 1851* (US); Cozumel Is., Bay of Honduras, *Gaumer in 1886*, TYPE collection var. *gracilis* (US). WEST INDIAN ISLANDS: South Caicos, Caicos Islands, Bahamas, *Wilson 7671* (G, M); near Nassau, New Providence Is., Bahamas, *Curtiss 89* (G, M, P, US); Trinidad, *Broadway 6660* (M, US); St. Croix, Dutch West Indies, *Ricksecker 297* (C, G, M, US); Palmarito, Prov. Santiago, Cuba, *Pollard and Palmer 316* (C, G, M, US); Santiago de Las Vegas, Prov. Havana, Cuba, *van Hermann 530* (CA, M, US). ARGENTINA: Cordoba, *Parodi 7477* (G, probably introduced).

19. N. SCHAFFNERI Gray ex Hemsl. Biol. Cent.-Am. Bot. **2**: 361. 1882; Brand, l. c. 148. *Marilaunidium Schaffneri* (Gray) Kuntze, l. c. Pl. 27, fig. 30.

A spreading, very leafy, strigose-hirsute annual (?), the branches somewhat ascending, 5–15 cm. long; leaves oblong, oblong-lanceolate, or spatulate, 2–6 cm. long, 3–7 mm. broad, sessile and somewhat clasping, or with winged petioles, the wings pseudo-clasping; flowers borne singly or in 2's on slender pedicels 2–10 mm. long; calyx-lobes narrowly linear-lanceolate, 5–7 mm. long; corolla narrowly tubular-campanulate, 8–12 mm. long; stamens unequally inserted ca. 2 mm. from corolla-base, free filaments filiform, 3–4 mm. long, adnate portion widened, the margins free to base of corolla, forming scales some of which often project as free tooth as much as 1 mm. above point of stamen insertion; styles 3–4 mm. long; capsules about 150-seeded; seeds light yellow, ellipsoid, ca. 0.3 mm. long, shallowly alveolate, the pits in more or less regular longitudinal rows, 5–6 per row, markings much like those of *N. biflorum* but less prominent.

Material seen—MEXICO: San Luis Potosi—22° N., *Parry and Palmer 609* (G, M, US), *Schaffner 395 in 1879* (CA, US), near Morales, *Schaffner 194 in 1876* (G, TYPE). Saltillo, Coahuila, *Gregg in 1848*, with some doubt (M).

20. N. UNDULATUM H. B. K. Nov. Gen. et Spec. Pl. **3**: 130. 1818; Choisy in DC. Prodr. **10**: 182. 1846; Gray, Proc. Am. Acad. **5**: 338. 1861; Brand, l. c. 156. *N. undulatum* var. *hispidum* (Griseb.) Brand, l. c. *Marilaunidium undulatum* (H. B. K.) Kuntze, l. c. *N. echioides* Grisebach, König. Ges. Wiss. Gött. Abh. (Pl. Lorent.) **19**: 230. 1874. *N. echioides* var. *hispida* Griseb. Symb. Fl. Argent. 267. 1879. *Marilaunidium echioides* (Griseb.) Kuntze, Rev. Gen. Pl. **3²**: 203. 1893. *Hydrolea congesta* Willd. ex Roem. et Schult. Syst. **6**: 192. 1820. *Hydrolea rupincola* and *H. radians* Moc. and Sesse ex Choisy, Mém. Soc. Phys. Genève **6**: 112. 1833. Pl. 27, fig. 10 and 15.

A prostrate or ascending, villous-hirsute and somewhat mealy-glandular annual, the branches several from the base, rather stout, sparingly branched; leaves oblong-lanceolate or oblong to spatulate, 1.5–5 cm. long, 2–12 mm. broad, undulate, the basal and lower cauline ones spatulate and attenuate to a broad petiole, upper cauline ones sessile and clasping, the midrib of leaf often somewhat decurrent on stem; flowers congested in numerous lateral or apical leafy, few-flowered cymes, on pedicels 1–3 mm. long; calyx-lobes linear-spatulate, 3–5 mm. long in flower, 5–10 mm. long in fruit; corolla narrowly obconic-campanulate, 6–9 mm. long; stamens unequally inserted 1–1.5 mm. from base of corolla, free portion of filament terete, widened slightly at juncture with corolla, adnate base thickened and widened, especially at top, the margins free almost to base of corolla; styles 2–4 mm. long; capsule 5–7 mm. long, 100–180-seeded; seeds pale yellow, ca. 4 mm. long, nearly ellipsoid, shallowly reticulate.

Representative material—UNITED STATES: mouth of Tarlinga R., Texas, *Havard 95* (G). MEXICO: Victoria, Tamaulipas, *Runyon 823* (US), *Palmer 143* (G, M); San Miguel between Laredo and Bejar, *Berlandier 1435=175* (M, but not G); Monclova, Coahuila, *Palmer 858* (G, US); near Chihuahua, *Pringle 267* (C, G, M, US); Colonia Juarez, Sierra Madre Mts., Chihuahua, *Jones* in 1903 (P, S); 5 mi. below Minas Nuevas, Sonora, *Rose, Standley and Russell 12676* (US); vic. of Guadalupe, Sinaloa, *Rose, Standley and Russell 14666* (US); Durango and vicinity, *Palmer 156* (C, G, M, US); San Luis Potosi, *Schaffner 397* in 1879 (CA, US), *Parry and Palmer 607* (G, M, US); mts. of Santa Rosa, Guanajuato, *Dugès* in 1901 (G); near Tula, Hidalgo, *Rose, Painter and Rose 8338* (US), *Pringle 7585* (P); Guadalajara, Jalisco, *Pringle 9379* (G, US); San Juanito, Oaxaca, *Conzatti 2173* (G). CHILE?: Andes of Chile, ex herb. *Pavon* (G). ARGENTINA: Andalgalá, Prov. Catamarca, *Jörgensen 1052* (C, G, M); Prov. Cordoba, *Lossen 2* (G, M); Rio Sali, Dept. Capital, Prov. Tucuman, *Venturi 914* (CA, G); Nonogasta, Dept. Chilecito, Prov. La Rioja, *Venturi 7823* (G).

20a. N. UNDULATUM var. MACRANTHUM Choisy, Mém. Soc. Phys. Genève 6: 112, pl. II, fig. 1. 1833; Gray in Hemsl. Biol. Cent.-Am. Bot. 2: 364. 1882. *N. macranthum* (Choisy) Brand, l. c. 153. *Marilaunidium macranthum* (Choisy) Kuntze, Rev. Gen. Pl. 2: 434. 1891. *N. Berlandieri* Gray, Proc. Am. Acad. 8: 282. 1870. *N. jamaicense × undulatum* Brand, l. c. 157.

Much more slender than the species, the leaves less numerous, clasping, margins slightly decurrent; flowers less numerous, the terminal cymes few, lax, flowers on pedicels as much as 1 cm. long; corolla 8–11 mm. long; seeds as in the species.

Material seen—MEXICO: Montemorelos, Nuevo Leon, *Nelson 6697* (G, US); Tamaulipas—*Berlandier* (M), Victoria, *Runyon 914* and *930* (US), *Berlandier 2215=795* (G); Jaumave Valley, *Nelson 4452* (US); near Reynosa, *Berlandier 2116=699*, TYPE collection (G, M, US); near Tampico, *Berlandier 2195=775* (G, M).

Although this aggregate is not a well marked variety, there does not seem to be adequate evidence for the assumption of hybridity, as the type of *N. Berlandieri* (which Brand called a hybrid) is identical with the plate of the var. *macranthum*.

20b. **N. undulatum** var. **australis** var. nov.

Very densely leafy, grayish-villous, the leaves strongly revolute, mostly not over 1.5 cm. long; calyx 6–7 mm. long; corolla 5–6 mm. long; capsules 4–5 mm. long, 20–100-seeded. Type: La Pampa, valley of Rio del Transito, Prov. Atacama, Dept. Vallenar, Chile, *Johnston 5867* (G). (Foliosissimum, incano-villosum; foliis revolutis, 1.5 cm. longis; calyce 6–7 mm. longo; corolla 5–6 mm. longa; capsules 4–5 mm. longa, 20–100-sperma. Type: La Pampa, valley of Rio del Transito, Prov. Atacama, Dept. Vallenar, Chile, *Johnston 5867* (G).)

Material seen: CHILE—above El Chivato, Rio de la Laguna Grande, e. of Vallenar, Prov. Atacama, Dept. Vallenar, *Johnston 5866* (G), and the type.

21. **N. HISPIDUM** Gray, Proc. Am. Acad. **5**: 339. 1861 (in large part); Hemsl. Biol. Cent.-Am. Bot. **2**: 364. 1882. *N. hispidum* var. *tenue* (Small) Brand, Pflanzenr. 4^{251}: 154. 1913. *Marilaunidium tenue* Small, Bull. Torr. Bot. Club **25**: 142. 1898.

A strigose-hirsute to hispid annual, simple at base, sparingly branched above, or if branched at base, the branches not spreading, the plant strict, erect, slender, 10–40 cm. tall; leaves variable, linear to linear- or oblong-spatulate, 1–5 cm. long, 1–5 mm. broad, tapering gradually to base, uniformly hispid to hispidulous, slightly revolute; flowers borne singly or in 3–5-flowered terminal cymes; calyx-lobes linear-lanceolate, 4–7 mm. long; corolla tubular-campanulate, 8–15 mm. long; stamens about half the length of corolla, filaments terete but rather thick, very unequally inserted 1–4 mm. from corolla-base, the adnate portion not much wider than free filament, the margins scarcely at all free; styles 2–4 mm. long; capsules 20–100-seeded; seeds ca. 0.5 mm. long, yellow, minutely alveolate-reticulate, the markings much like those of *N. undulatum* but more regular.

Representative material—UNITED STATES: Oklahoma—Arbuckle Mts., near Davis, *Emig 905* (M); vic. of Fort Sill, *Clemens 11741* (M). Texas—without locality, *Berlandier 2385 = 898*, TYPE collection (G, M), *Lindheimer 200* (M) and *130* (C, G, M); Dallas, Dallas Co., *Bush 669* (G, M); Lake Worth, Tarrant Co., *Ruth 886* (G); Comanche Spg., Comanche Co., *Lindheimer 1009* (C, G, M); Bastrop, Bastrop Co., *Tharp 2566* (P); Austin, Travis Co., *Tharp* in 1920 (P); Fredericksburg, Gillespie Co., *E. J. Palmer 10047* (M); Spanish Pass, Kendall Co., *Clemens and Clemens 312* (CA, M, P, S); San Antonio, Bexar Co., *Wilkinson 120* (M); Horseshoe Lake, Jackson Co., *Drushel 4114* (M); Corpus Christi, Neuces Co., *Heller 1461* (C TYPE collection of *M. tenue*, and the plant matches the description, but not G, M); Laredo, Webb Co., *Reverchon 3892* (G, M); Devils R., Valverde Co., *Orcutt 6228* (M); Dryden, Terrell Co., *Jones 25736* (P); exp. from W. Texas to El Paso, N. M., *Wright 493* (C, G). New Mexico—10 mi. w. of Santa Fe, *Heller and Heller 3737* (M). Colorado—*T. S. Brandegee 5277* (M, near the var. *spathulatum*). MEXICO: Colonia Diaz, Chihuahua, *Nelson 6436* (US); vic. of Chihuahua, *Palmer 378* (M, US, in part, approaching the var. *Mentzelii*); Sinaloa, Sinaloa, *Ortega 4540* (US); between Matamores and Giliad, Puebla, *Berlandier 2486 = 1056* (G, M).

The species is extremely variable and not easily separable into well-marked varieties. When Gray published the species, he included material of this entity in greatest part, *Berlandier 2385* being the first number cited. Since it is apparent that this is the group which Gray had in mind chiefly, it must be considered the species proper, and the aggregate which Brand treated as the species must be accorded varietal rank as follows.

21a. **N. hispidum** var. **spathulatum** (Torrey) comb. nov. *N. biflora* var. *spathulatum* Torr., Pac. R. R. Surv. Rep. 7³: 17. 1856. *N. hispidum* Gray, l. c. in small part; Brand, l. c. fig. 29. *Conanthus hispidus* (Gray) Heller, Bull. Torr. Bot. Club 24: 479. 1897. *Marilaunidium hispidum* (Gray) Kuntze, Rev. Gen. Pl. 2: 434. 1891. *N. jamaicense?* Engelm. and Gray, Pl. Lindh. 1. no. 130, non. Linn. (fide Gray). *N. dichotoma* Torr. Bot. Mex. Bound. Surv. 147. 1859, acc. spec. cited.

Plant branching from base, the branches ascending to erect, 5–30 cm. long; leaves 1–7 mm. long, 1–8 mm. broad, plane or somewhat revolute; otherwise as in the species.

Representative material—UNITED STATES: Texas—Comancheries Orientales, *Berlandier 506* (G); exp. from W. Texas to El Paso, N. M., *Wright 494* and *495* (C, G); Corpus Christi, Nueces Co., *Heller 1461*, TYPE collection of *M. tenue* but these plants do not match description as does the collection at Univ. of Calif. (G, M); El Paso, *Jones 3733* (C, CA, P, S); Quitman Mts., near Sierra Blanca, *Ferris and Duncan 2468* (CA, M, S). New Mexico—mesa w. of Organ Mts., D. A. Co., *Wooton* in 1905 (C, P, S); Mesilla, D. A. Co., *Wooton 27* (C, G, M, P, S); Santa Fe, *Fendler 643* (G, M); Animas Cr., Sierra Co., *Metcalfe 1145* (CA, G, M, P); Gila R. bottom near Cliff, Grant Co., *Metcalfe 143* (CA, G, M, P, S); 10 mi. w. of Deming, *Goodman and Hitchcock 1151* (C, M, S). Arizona—Ft. Lowell, *Thornber 452* (C, M), *W. F. Parish 157* (C, G, S); Yucca, *Jones 3902* (P, S); Camp Grant, *Palmer 188* (G, M). California—"Hayfields," Chuckawalla Valley, *Munz and Keck 4803* (P); Palo Verde Valley, *Jepson 5259* (S); Barstow, *Tracy and Earle 65* (C, G, M). MEXICO: Laredo, Tamaulipas, *Berlandier 1420 = 160* (G); Nuevo Leon—near Monterey, *Gregg 185* (M); Pico Chico, near Monterey, *Canby 169* (G, US). Coahuila—Rio Grande Valley near Diaz, *Pringle 9011* (G, M, US); Saltillo, *Palmer 84* (C, G, M, US); Parras, *Purpus 1874* (C, G, M, US). Chihuahua—without locality, *Hartman 643* (C, G, US); vic. of Aldama, *Palmer 238* (M, US). Sonora—Fronteras, *Thurber 448* (G); near Magdalena, *Rose, Standley and Russell 15084* (US). Baja California—San Vicente, *T. S. Brandegee* in 1893 (C), *Orcutt* in 1884 (M); Seven Wells, Salton R., *Schoenefelds 288* (G, S, US). Sinaloa—Sinaloa, *Goldman 310* (G, US, approaching the var. *sonorae*). Durango—Mapimi, *Pringle 87* (G); Valley of Nazas, *Gregg 440* (M). San Luis Potosi—Minas de San Rafael, *Purpus 4861*, unusually slender and flexuous-branched (C, G, US).

Material from the region of the Colorado River is usually characterized by softer pubescence and more revolute leaves, and may be distinguished as follows:

21b. N. HISPIDUM var. REVOLUTUM Jepson, Man. Fl. Pl. Calif. 832. 1925. *N. hispidum* Brand, Pflanzenr. 4²⁵¹: 153. 1913, in large part.

Leaves grayish, soft-hirsute as well as hispid, strongly revolute.

Representative material—UNITED STATES: Arizona—Yuma, *Beard* in 1911 (M). California—Imperial Co.: Colo. R. near Ft. Yuma, *Peirson 7199* (CA); Colo. R. at Pilot Knob, May 8, 1910, *Grinnell* (C TYPE); Mecca, *Munz and Keck 4740* (C, P); Colo. R. bottom near Ripley s. of Blythe, *Ferris 7188* (C, S); Chuckawalla Pass, *Bailey and Bailey* in 1927 (G); "Hayfields," Chuckawalla Valley, *Munz and Keck 4931* (C, P). San Diego Co.—Calexico, *Davy 7965* (C, M). MEXICO: Sonora—El Alamo, near Magdalena, *Kennedy 7109* (C, CA, US). Baja California—Seven Wells on the Salton R., *Mearns 2860* (US).

To this group belongs much of the material cited by Brand under his var. *Coulteri,* which is not the same as the true *N. Coulteri* of Gray.

21c. **N. HISPIDUM** var. **MENTZELII** Brand, l. c. 155. *N. hispidum* var. *Coulteri* Brand, l. c. 154, in small part. *Marilaunidium foliosum* Woot. and Standl. Contr. U. S. Nat. Herb. **16**: 162. 1913.

Leaves obovate-elliptic, obovate-spatulate, or obovate, ca. 12 mm. long, 2–4 (6) mm. broad, the margins and upper surface hirsute-hispid, the lower surface glandular, with hirsute pubescence only on midrib, or none at all; corolla 7–9 mm. long.

Representative material—UNITED STATES: Texas—near Ft. Davis, Jeff Davis Co., *Ferris and Duncan 2712* (CA, M, S), *E. J. Palmer 32081* (M); mouth of St. Helena Canyon, Brewster Co., *Moore and Steyermark 3461* (M); San Angelo, Tom Green Co., *Palmer 12388* (C). New Mexico—near Roswell, Chaves Co., *Earle 531,* TYPE of *M. foliosum* (US), *558* (M), *Earle and Earle 531* (P), *Earle and Earle 558* (M). MEXICO: Rio Grande, *Berlandier 2443 = 1013* (G); Colonia Juarez, Sierra Madre Mts., Chihuahua, Sept. 11, 1903, *Jones* (P); Coahuila-Saltillo, *Palmer 84½* (C, G, M, US); s. of Parras, *Palmer 859* (G, US); Diaz, *Rose and Hay 5257* (US).

21d. **N. hispidum** var. **sonorae** var. nov.

Plant rather small, soft-pubescent, branches 3–12 cm. long, sparingly villous-hirsute, glandular; sepals linear-spatulate, ca. 6 mm. long; corolla 12–15 mm. long. TYPE: Torres, Sonora, Mexico, *Coville 1663* (US herb. no. 398086). (Humile, molli villosum; ramulis 3–12 cm. longis, glandulosis; sepalis lineario-spathulatis, ca. 6 mm. longis; corolla 12–15 mm. longa. Torres, Sonora, Mexico, *Coville 1663* [US herb. no. 398086 TYPE].)

Material seen—MEXICO: Sonora—Guaymas, *Palmer 172* (CA, G, US) and the type.

22. **N. Stevensii** C. L. Hitchcock sp. nov. *Nama compactum* Stevens ex Jeff and Little, Publ. Univ. Okla. Biol. Surv. **2**[2]: 77. 1930 (herbarium name published as a nomen nudum). Pl. 27, fig. 27.

A grayish-strigose-hispid, erect, few-branched annual, 5–25 cm. tall; leaves linear-lanceolate, much acuminate, 1–3 cm. long, 1–3 mm. broad, revolute, sessile, somewhat clasping; flowers sessile and axillary in leafy terminal clusters; calyx-lobes much acuminate, linear-lanceolate, 5–8 mm. long; corolla tubular, ca. 8 mm. long; stamens not more than half as long as corolla, very unequally inserted 1–3 mm. from corolla-base, filaments terete, 1–2 mm. long, the adnate bases widened, margins free, thus filaments with 2 narrow divergent scales running from base; styles ca. 4 mm. long; capsules 40–50-seeded; seeds yellow, 0.3 mm. long, alveolate. TYPE: bare top of red clayey hill, near Alva, Woods Co., Oklahoma, May 27, 1913, *Stevens 665* (Stanford herb. no. 65741). (Annuum, albido-strigoso-hirsutum, erectum, parce ramosum, 5–25 cm. altum; foliis lineario-lanceolatis, acuminatissimis, 1–3 cm. longis, 1–3 mm. latis, revolutis, sessilibus; floribus in capitulis terminalibus sessilibus vel axillariis; sepalis acuminatissimis, lineario-lanceolatis, 5–8 mm. longis; corolla cylindrico-infundibuliforma, ca. 8 mm. longa; staminibus inaequalibus, cum corolla 1–3 mm. supra basi coalitis, ad basem amplificatibus, marginibus non connatis; stylis non connatis, ca. 4 mm. longis; capsula 40–50-sperma, semina laete brunnea, 0.3 mm. longa, alveolata. TYPE: bare top of red clayey hill, near Alva, Woods Co., Oklahoma, May 27, 1913, *Stevens 665* [Stanford University herb. no. 65741].)

Material seen—UNITED STATES: Oklahoma—Woods Co., *White 8* (M); bare top of red clayey hill, near Alva, Woods Co., May 27, 1913, *Stevens 665* (G, S TYPE); near Cora, Woods Co., *Stevens 736* (G, S); near Fairvalley, Woods Co., *Stevens 716* (G, S); near Waynoka, Major Co., *Stevens 590* (G, M, S); Woodward Co., *White 19* (M). Texas—Sweetwater, Nolan Co., *Reverchon 1241* (M, intermediate in character between this species and *N. hispidum*).

Nama Stevensii is most closely related to *N. hispidum,* but differs in habit and leaf character as well as in the nature of the filament bases. It is somewhat similar to *N. stenophyllum* in habit, but need not be confused with that species, which is a perennial.

23. N. COULTERI. Gray, Proc. Am. Acad. **8**: 283. 1870, Johnston, Proc. Cal. Acad. Sci. IV, **12**: 1135. 1924. *N. hispidum* var. *Coulteri* (Gray) Brand, Pflanzenr. 4 ^{251}: 154. 1913, in part. *N. parvifolium* var. *brevistylum* Brand, l. c. 155. *Conanthus Coulteri* (Gray) Heller, Cat. N. Am. Pl. 6. 1898. *Marilaunidium Coulteri* (Gray) Kuntze, Rev. Gen. Pl. **2**: 434. 1891.

A short-villous-hirsute, ascending, slender, branched annual, the branches filiform, 5–30 cm. tall; leaves obovate to obovate-spatulate or spatulate, 1–3 cm. long, 3–8 mm. broad, gradually attenuate to base; flowers mostly single in the axils, subsessile; calyx-lobes linear-spatulate, 4–7 mm. long (longer in fruit); corolla 7–10 mm. long, obconic-campanulate; stamens about half the length of the corolla, unequally inserted 1–2 mm. from base, filaments filiform, the adnate bases widened considerably, with free margins; styles 2–4 mm. long; capsules 20–80-seeded; seeds yellow, ca. 0.4 mm. long, alveolate-reticulate, like those of *N. hispidum.*

Material seen—MEXICO: Baja California—*Coulter 643* (G TYPE, label says collected in Calif., but more probably collected in Baja California); Calmalli, *Purpus 200,* TYPE collection *N. parvifolium* var. *brevistylum* (C, P, US); near tule swamp, Mulege, *Johnston 3674* (G, US); San Gregorio, Feb. 1, 1889, *T. S. Brandegee* (C); Santa Agueda, *Palmer 240* (C); Santo Domingo, *Purpus 114* (C); La Paz, *Collins, Kearney and Kompton 67* (US), *Jones 24064* (M, P); San Jose del Cabo, *Anthony 348* (G, M, S, US), *T. S. Brandegee,* March 15, 1892, and in 1897 (C), *Rose 16486* (US). Sonora—Torres, *Purpus 413* (C, M, US); vic. of Hermosilla, *Rose, Standley and Russell 12365* (US); 5 mi. below Minas Nuevas, *Rose, Standley and Russell 12675* (US); Las Durasnillas, May 18, 1892, *T. S. Brandegee* (C). Sinaloa—vic. of Fuerte, *Rose, Standley and Russell 13471* and *13539* (US); vic. of San Blas, *Rose, Standley and Russell 13228* and *13410* (US), *Jones 23147* (C, CA, M, P), *23523* (P).

Nama Coulteri resembles *N. hispidum* rather closely in most characters, but differs in the more soft pubescence, more filiform branching, and wider free-margined filament bases. Brand's var. *Coulteri* contained plants of true *Coulteri* as well as many numbers that are really *N. hispidum.* On the mainland of Mexico, the species grades into the following variety:

23a. **N. Coulteri** var. **Pringlei** (Rob. and Greenm.) comb. nov. *N. Pringlei* Rob. and Greenm. Proc. Am. Acad. **32**: 38. 1896; Brand, l. c. 149.

Leaves ovate to oblong, 5–10 mm. broad, with slender petioles.

Material seen—MEXICO: Puebla—near Tehuacan, *Pringle 9515* (G, M, US) and *6286,* TYPE collection (C, CA, G, M, US), *Rose and Hay 5867* and *5868* (US).

24. N. SANDWICENSE Gray, Proc. Am. Acad. **5**: 338.   1861 ; Brand, l. c. 152.   *Conanthus sandvicensis* (Gray) Heller, Minnesota Bot. Stud. **1**: 879. 1897.   *Marilaunidium sandwicensis* (Gray) Kuntze, l. c. *N. sandwicense* var. *laysanicum* Brand, l. c. judging from characters in description.   Pl. 27, fig. 24.

An ascending, loosely but freely branched, hirtellous, cinereous annual (?), the branches 5–25 cm. long; leaves spatulate, 6–15 mm. long, 3–7 mm. broad, strongly revolute; flowers scattered, singly or in 2's, pedicels 2–5 mm. long; calyx-lobes narrowly linear-oblong, slightly larger above middle, ca. 4 mm. long; corolla 5–8 mm. long, narrowly obconic; stamens unequally inserted about 1 mm. from corolla-base, filaments filiform, adnate portion with narrow free edge just below point of stamen insertion; styles 1–2 mm. long; capsules 20–60-seeded; seeds ca. 0.5 mm. long, light brown, oblong-ellipsoid, shallowly alveolate-pitted, markings much like those of *N. biflorum*.

Material seen—HAWAIIAN ISLANDS: *Wilkes Expl. Exp.* in 1838–42 (G, US) ; Woahoo, *Macrae* in 1825 (G) ; Oahu, *Remy 425* (G), *Nuttall* (G), *Mann and Brigham 97*, TYPE collection (G, M, US), *Heller 1956* (US), *Degener 2067* (US).

25. N. PARVIFOLIUM (Torr.) Greenman, Proc. Am. Acad. **39**: 85.   1904; Brand, l. c. 155.   *N. dichotoma* var. *parvifolia* Torr., Bot. Mex. Bound. Surv. 147.   1859.   *N. rupicola* as treated by Gray, Proc. Am. Acad. **8**: 284.   1870, in part (*Schott* specimen).   *N. rupicolum* as treated by Gray in Hemsl. Biol. Cent.-Am. Bot. **2**: 362.   1882, in part (*Eaton and Edwards* specimen).   *N. origanifolium* as treated by Gray, Proc. Am. Acad. **5**: 337.   1861, in large part, as to specimens cited.   *N. humilis* Nees, ex Brand, l. c. (publ. in synonymy).

A slender, ascending, somewhat cinereous, short-villous-hirsute annual, branches weak, slender, 5–20 cm. long; leaves rather thin, spatulate to obovate-oblong, 7–15 mm. long, 2–6 mm. broad, with narrowly-winged petioles 1–4 mm. long; flowers borne singly or in 2's, pedicels slender, 3–6 mm. long; calyx-lobes linear-lanceolate, 4–6 mm. long; corolla 7–10 mm. long; stamens extending slightly beyond middle of corolla, very unequal, unequally inserted 1–3 mm. from base of corolla, filaments filiform, the adnate bases widened somewhat, with very narrow free-margins running nearly to base of corolla; styles ca. 5 mm. long; capsules with 60–80 seeds; seeds brown, ca. 0.3 mm. long, shallowly alveolate-pitted and minutely transversely corrugated, like the seeds of *N. torynophyllum*.

Material seen—MEXICO: San Fernando to Jimeney, Tamaulipas, *Nelson 6606* (G, US) ; valley of Rio Grande near Piedras Negras, Coahuila, *Pringle 9173* (G, US) ; Soledad, Coahuila, *Palmer 2023* (G) ; Sta. Rosa, Chihuahua, *Bigelow* (G, TYPE) ; Bishop's Hill, near Monterey, Nuevo Leon, *Gregg 184* (M) ; Monterey, Nuevo Leon, *Edwards and Eaton* (G) ; YUCATAN : *Schott* (G, wrong locality ?).

Nama parvifolium has been confused with *N. rupicolum* and *N. origanifolium*, but the leaves and seeds are different, the corolla is larger, and the stamens are inserted unequally and have winged adnate bases.   Furthermore, the latter species is a perennial.   It would seem that *N. parvifolium* is much more closely related to *N. rotundifolium*, but it is not villous, the leaves are thicker than in that species, the corollas are larger, and the filament-bases are different.

26. N. PROSTRATUM Brand, Pflanzenr. 4²⁵¹: 148. 1913. Pl. 27, fig. 28.

A creeping or scandent, hispid, leafy annual; branches as much as 8 cm. long; leaves ovate-rotund to obovate, 8–20 mm. long, 6–12 mm. broad, distinctly petiolate; flowers terminal, solitary or in 2's, on pedicels 2–3 mm. long (longer?); calyx-lobes spatulate, ca. 4 mm. long; corolla infundibuliform, ca. 1 cm. long; stamens subequally inserted ca. 2 mm. from corolla-base, filaments slightly widened just above point of adnation, the adnate bases slightly widened, with free margins for about 1 mm.; styles ca. 7 mm. long; ovary ca. 20-seeded; mature seeds not seen.

Material seen—MEXICO: Temascaltepec, Oaxaca, *Ehrenberg 326*, fragment of TYPE (P).

Nama prostratum is undoubtedly most closely related to *N. parvifolium* but differs in several characters, and especially because of the larger leaves and the larger corolla.

27. N. DICHOTOMUM (Ruiz and Pavon) Choisy, Mém. Soc. Phys. Genève 6: 113. 1833; Gray, Proc. Am. Acad. 5: 338. 1861; Brand, l. c. 150. *Hydrolea dichotoma* Ruiz and Pavon, Fl. Peruv. 3: 22. pl. 244, fig. b. 1802. *N. tetrandra* Pavon ex Choisy, l. c. (publ. in synonymy). *N. stricta* Phil. Fl. Atac. 37. 1860. *Marilaunidium strictum* (Phil.) Kuntze, Rev. Gen. Pl. 2: 434. 1891. *N. dichotomum* subsp. *eu-dichotomum* Brand, l. c. 151 (in part). *N. dichotomum* subsp. *eu-dichotomum* var. *stricta* (Phil.) Brand, l. c. 151, fig. 28. *N. dichotomum* var. *angustifolium* Gray, Proc. Am. Acad. 8: 284. 1870. *N. angustifolium* (Gray) A. Nels., Coult. and Nels. New Man. Rcky. Mt. Bot. 410. 1909. *N. dichotomum* subsp. *angustifolium* (Gray) Brand, l. c. *N. dichotoma* β *pauciflora* Choisy ex Gray, Proc. Am. Acad. 8: 284. 1870. *Conanthus angustifolius* (Gray) Heller, Bull. Torr. Bot. Club 24: 479. 1897. *Marilaunidium dichotomum* (R. and P.) Kuntze, l. c. *Marilaunidium tenue* Woot. & Standl. Contr. U. S. Nat. Herb. 16: 162. 1913. Pl. 27, fig. 1 and 29.

An erect or ascending, simple or semi-dichotomously branched, hirtellous or hirsute, glandular annual, 7–20 cm. tall, branches with few leaves, the nodes usually 1–5 cm. apart; leaves linear-elliptic, linear-oblanceolate, or narrowly spatulate, 5–30 mm. long, 1–4 mm. broad, narrowed to short petiole; flowers borne singly (2's) in the upper dichotomous branches on pedicels 1–3 mm. long; calyx-lobes linear or linear-spatulate, ca. 4 mm. long in flower, increasing in length to as much as 10 mm. in fruit; corolla tubular-campanulate, ca. 5 mm. long; stamens unequally inserted scarcely 1 mm. from corolla-base, the filaments filiform, widened and flattened just above the adnate base, the adnate portion with delicate, free margins that run nearly to base of corolla; styles 0.5–2 mm. long; capsules 20–60-seeded; seeds brown, ca. 0.5 mm. long, the surface uniformly minutely alveolate as well as large-pitted.

Representative material—UNITED STATES: Colorado—Fremont Co., *T. S. Brandegee 1046* (C, G); 8 Mile Park, *T. S. Brandegee 5275* (M); New Mexico— without locality, *Fendler 644*, TYPE collection var. *angustifolium* (G, M); Santa Fe Canyon, 9 mi. e. of S. Fe, *Heller 3846* (G, M); Mogollon Mts., near w. fork of Gila R., Socorro Co., *Metcalfe 675* (M), *Rusby 274* (M); near Pecos, San Miguel Co., *Standley 5056* (G, M); 3 mi. s. of Hillsboro, Black Range, Sierra Co., *Metcalfe 1291*, TYPE collection *M. tenue*

(CA, G, M, P, US) ; Arizona—near Flagstaff, *Hanson and Hanson. A934* and *A955* (M) ; May–Oct. 1901, *Purpus* (C, M) ; Rincon Mts., *Blumer 3370* (C, G, S) : MEXICO: without locality, *Coulter 916* (G), *Orcutt 4134* (M) ; mts. near Chihuahua, *Pringle 775* (C, G, M, US) ; hills near Guerrero, Chihuahua, *Pringle 1335* (US) ; Soldier Canyon, Sierra Madre Mts., Chiihuahua, Sept. 16, 1903, *Jones* (P) ; s. of Saltillo, Coahuila *Palmer 855* (G, US) ; Durango and vic., *Palmer 698* (G, US) ; Tejamen, Durango, *Palmer 500* (C, M) and *501* (G, US) ; San Luis Potosi, 22° N., *Parry and Palmer 610* (M in part, G, US) ; Alvarez, S.L.P., *Orcutt 1761* and *1659* (US) ; Federal District— Xochimilco, *Orcutt 4337* (M) ; Eslava, Valley of Mexico, *Pringle 9362* (G, M, P, US), *Rose and Painter 7146* (US) ; top of El Penon, Valley of Mexico, *Rose and Hay 5969* (G, US) ; Puebla—Boca del Monte, *Purpus 5761* (C) and *5661* (G, M, US) ; Oaxaca— Teposcolula, *Seler 1554*, approaching the var. *chasmogamum* (G). ECUADOR: without locality *Hartweg 1239* (G) ; in Andibus Ecuadorensibus, *Spruce 5802* (G). PERU: Ambo, *Macbride 3192* (G) ; Ollamtaitambo, Dept. Cusco, *Pennell 13679* (G) ; San Bartolome, *Weberbauer 5291* (G) ; Arequipa, Dept. Arequipa, *Pennell 13054* (G) ; Tingo, Dept. Arequipa, *Pennell 13108* (G) ; s. slopes of Chachani Mt., n. of Arequipa, *Hinkley 74* (G). CHILE: Paposo, *Philippi*, fragment of TYPE of *N. strictum* (G). GALAPAGOS ISLANDS: Albermarle I., Tagus Cove, *Stewart 3144* (CA).

27a. **N. DICHOTOMUM** var **CHASMOGAMUM** Brand, l. c. *N. campanulatum* Brand l. c. 152.

Plant much more robust and more profusely branched than the species, 15–50 cm. tall, minutely hirtellous ; leaves oblong-elliptic to oblong-lanceolate, 1–4 cm. long, 2–12 mm. broad, with slender petioles ; flowers more numerous than in the species, 1–3 at the nodes and in axillary and terminal lax cymes, pedicels slender, 2–6 mm. long ; corolla 4–7 mm. long ; styles 1.5–6 mm. long.

Representative material—MEXICO: San Luis Potosi—Alvarez, *Palmer 170* (G. M, US) ; near Los Canoas, *Palmer 244* (US), *Pringle 3312*, TYPE collection (C, G, M, P, US) ; El Salto Station, Hidalgo, *Pringle 11638* in part (G, US) ; Borrego, region near Orizaba, Vera Cruz, *Bourgeau 3163* (G) ; Chiapas, *Ghiesbreght 614* (G, M) ; near Zimapan, *Aschenborn 208*, fragment of TYPE of *N. campanulatum* (P).

The status of *N. campanulatum* is not definitely established, but judging from Brand's description and the fragment of the type at Pomona, it seems certain that the plant is some form of *N. dichotomum*. However, Brand says the seeds are reticulate. The seeds on the fragment of the type are not mature, so that this point cannot be settled.

27b. **N. DICHOTOMUM** var. **PUEBLENSE** (Robins. and Greenm.) Macbride, Contr. Gray Herb. **49**: 45. 1917. *N. pueblense* Robinson and Greenman, Proc. Am. Acad. **32**: 39. 1896. *N. dichotomum* var. *eu-dichotomum* Brand, l. c. 151 (in large part).

Leaves ovate or oblong-ovate to obovate, 1–3 cm. long, 3–15 mm. broad, with slender petioles 3–8 mm. long ; pedicels 1–5 mm. long ; calyx and leaves grayish-hirsute ; capsules 10–30-seeded.

Representative material—MEXICO: Chojo Grande, 27 mi. s. of Saltillo, Coahuila, *Palmer 453* (US) ; reg. of San Luis Potosi, *Parry and Palmer 611* (G, M, US) ; vic. of Chihuahua, *Palmer 378* (US in part, but not M) ; near Higuerillas, Queretaro, *Rose, Painter and Rose 9795* (US) ; Ixmiquilpan, Hidalgo, *Purpus 404* (M, P, US) and *8898* (US) ; near Tehuacan, Puebla, *Pringle 9514* (G, M, US), *7429* (P). and *6287*, TYPE collection (C, CA, G, M, US), *Rose, Painter and Rose 10170* (US) ; San Luis Tultit-

lanapa, *Purpus 5005* (C) ; vic. of San Luis Tultitlanapa near Oaxaca, *Purpus 2511* (C, G, M, US) ; El Riego, Puebla, *Purpus 1287* (C) and *1278* (G, M, P).

27c. **N. dichotomum** var. **latisepalum** (Loesener) comb. nov. *N. dichotomum* f. *latisepala* Loesener, Bull. Herb. Boissier **7** : 568. 1899. *N. latifolia* Gray, Proc. Am. Acad. **8** : 284. 1870; Brand, l. c. 152. *Marilaunidium latifolium* (Gray) Kuntze, Rev. Gen. Pl. **2** : 434. 1891.

Delicate, ascending, sparsely hirtellous or hirsute, 5–20 cm. tall; leaves oblong-ovate, ovate, or ovate-lanceolate, 1–3 cm. long, 3–12 mm. broad, with slender pedicels about half as long as blades; flowers in 2-several-flowered lax cymes, on filiform pedicels 1–5 mm. long; calyx-lobes spatulate, well widened at apex ; flowers 3–4 mm. long.

Material seen—MEXICO: Tlaxiaco, Oaxaca, *Seler 1463*, TYPE collection (G) ; Oaxaca, *Galeotta 1068*, TYPE of *N. latifolium* (G) ; Puebla, *Seler 3627* (G).

There is very little basis for maintaining this aggregate as a species, the wider sepals and leaves being the only distinctive characters. The var. *hispidum* of Gray probably is not maintainable as an entity, but no material has been seen that could be referred to his variety.

27d. **N. dichotomum** var. **amplifolium** (Brand) comb. nov. *N. dichotomum* subsp. *eu-dichotomum* f. *amplifolia* Brand, l. c.

Rather simple, erect, hirsute; leaves ovate-lanceolate to oblong-elliptic, 1–3.5 cm. long, 4–10 mm. broad; flowers rather few, the pedicels 1–10 mm. long; calyx-lobes spatulate; corolla ca. 4 mm. long; styles 0.5–2 mm. long.

Material seen—ARGENTINA: La Junta, prov. Catamarca, Dept. Andalgalá, *Jörgensen 1728* (G, M). BOLIVIA: vic. Glorata, pr. Calavaya, *Mandon* in 1858 (G) ; Cochabamba, *Bang 958* (G, M). ·

28. N. DEMISSUM Gray, Proc. Am. Acad. **8** : 283. 1870; Wats. Bot. King Exp. 259. 1871 ; Brand, l. c. 159. *Marilaunidium demissum* (Gray) Kuntze, l. c. *Conanthus demissus* (Gray) Heller, l. c. Pl. 27, fig. 7.

A prostrate, sparingly divaricately branched, strigose and villous-hirsute annual, branches 3–15 cm. long, slender, with very long internodes, the leaves practically restricted to compact clusters at the ends of the branchlets; leaves obovate to spatulate or linear-spatulate, 1–2.5 cm. long, 1–7 (averaging about 3) mm. broad, usually gradually narrowed to petiole nearly as long as blade; flowers solitary in branch-axils and numerous in the leafy branch-tips, subsessile; calyx-lobes narrowly linear-lanceolate, grayish-villous-hirsute, 5–8 mm. long; corolla tubular-campanulate, 9–15 mm. long; stamens subequally inserted 2–3 mm. from base of corolla, free filaments terete, about equal to the slightly widened, free-margined adnate portion, the free margins of nearly uniform width and running nearly to base of corolla; styles 3.5–5 mm. long; capsules ca. 4 mm. long, 8–18-seeded; seeds brown, 0.6 mm. long, ovoid-obloid, irregularly pitted-canellate and very minutely transversely corrugated.

Representative material—UNITED STATES: Utah—Beaverdam, Box Elder Co., *Jones 5024aj* (P) ; Stansbury I., Tooele Co., *Watson 888* (G) ; St. George, Washington Co., *Parry 185* (G, M), *Jones 1653* (C, CA, P, S) ; Nevada—Rhyolite, Nye Co., Apr. 25, 1907, *Jones* (P) ; Clark Co., Moapa, *Kennedy 1843* (G, S), Las Vegas, *Goodding 2331* (C, G, M) ; Muddy Valley, Lincoln Co., *Kennedy and Goodding* in 1906 (C) ; Rhodes,

Mineral Co., June 22, 1882, *Jones* (P); Hawthorne, Mineral Co., June 23, 1882, *Jones* (P). California—Eagle Mts., Riverside Co., Mar. 24, 1926, *Jaeger,* somewhat intermediate in character (P); Kelso, San Bernardino Co., *K. Brandegee* in 1915 (P). The following collections are somewhat intermediate in character between the species and the variety *deserti:*—near St. George, Washington Co., Utah, *Cottam, Stanton and Harrison 4084* (P); Tucson, Pima Co., Ariz., *Lemmon 166* (C, G); Santa Rosa to Casa Grande, Ariz., *Griffiths 4032* (M); Moapa, Clark Co., Nev., *Goodding 2204* (C, G, M); near Lavic, San Bernardino, Calif., *Munz, Harwood and Johnston 4160* (P); Dos Palmos Spg., Riverside Co., Calif., *Munz 9959* (P).

Material of the species, proper, differs from most of the material from southern California, which has always been called *N. demissum,* because of the broader leaves, a character which is remarkably constant.

28a. N. DEMISSUM var. COVILLEI Brand, l. c. 159. *Marilaunidium demissum* (Gray) Coville, Contr. U. S. Nat. Herb. 4: 162. 1893, in part.

Plant gray-villous; leaves rhombic-obovate, 1-2 cm. long, 3-6 (averaging nearly 4) mm. broad.

Material seen—UNITED STATES: California—Inyo Co.: Funeral Mts., *Coville and Funston 453* (US, TYPE), Apr. 8, 1907, *Jones* (P); Furnace Cr., *Parish 10065* (G, S), *Howell 3678* (CA); Cave Spring, Mar. 12, 1924, *Jones* (P); Ryan, *Shockley* in 1908 (S); Salt Creek Crossing, *Ferris 4045* (S); between Stewart's Valley and Shoshone, *Ferris 7368* (S); 34 mi. n. of Baker, San Bernardino Co., *Howell 3597* (CA). The following collections are more or less intermediate between the variety *Covillei* and the variety *deserti:* Emigrant Spgs., Inyo Co., *Parish 10198* (C, G, S); Mill Creek Canyon, Panamint Mts., Inyo Co., *Coville and Funston 758* (G, S); Randsburg, Kern Co., *Heller 7701* (C, G, M, S).

28b. N. DEMISSUM var. DESERTI Brand, l. c. (char. emend.).

Pubescence and general aspect of the species, but leaves linear to narrowly spatulate, 1-4 cm. long, 1-3 (averaging about 1-1.5) mm. broad.

Representative material—UNITED STATES: Arizona—Congress Junction, Yavapai Co., May 2, 1903, *Jones* (P); Coyote to Santa Rosa, Pima Co., *Griffiths 4000* (M); Yucca, Mohave Co., May 13, 1884, *Jones* (P); Chimehuevis, Apr. 23, 1903, *Jones* (P). Nevada —10 mi. above Rioville, Apr. 11, 1894, *Jones* (P). California—Inyo Co., w. of Bishop, *Heller 8280* (C, CA, G, M, S); Great Falls Canyon, Argus Mts., *Bailey and Robison* in 1930 (M); San Bernardino Co., near Bonanza, Providence Mts., *Munz and Harwood 3465* (P); Kramer, *Parish 9765* (C, G, S); 10 mi. e. of Daggett, *Munz and Harwood 3669* (P); Victorville, Mar. 27, 1907, *Grinnell* (US, TYPE, a very depauperate specimen and thus really atypical of this variety), *Munz 2543* (P, S); Whiskey Spg., Cushenbury Canyon, May 12, 1926, *Jones* (C); Hesperia, *Parish 4995* (P, S); Riverside Co., Cottonwood Spgs., Apr. 12, 1924, *Evermann* (CA, M); Yaqui Wells, *Eastwood 2635* (CA, G); Palm Springs, *Parish 4128* (C, G, M); Iron Well Mts., Apr. 7, 1901, *T. S. Brandegee* (C); Coachella, *Greata 412* (C, S); "Hayfields," Chuckawalla Valley, *Munz and Keck 4931* (P); Kern Co., Red Rock Canyon, *Abrams 11862* (P, S); Los Angeles Co., Antelope Valley, Apr. 6-8, 1917, *Shaw, Spalding and Walton* (C, P); Palmdale, *Elmer 3619* (CA, M, P, S); Lancaster, *Davy 2289* (C); San Diego Co., San Felipe Wash, *Keck and McCulley 78* (P); 39 mi. w. of Mexicali, *Munz 9592* (P).

28c. **N. demissum var. linearis var. nov.**

Leaves linear, 1-3 cm. long, 0.5-2 (averaging not more than 1) mm. broad; calyxes gray-silky-pilose. TYPE: Baja California, San Gregorio,

Feb. 2, 1889, *T. S. Brandegee* (Gray Herbarium). (Folia linearia 1–3 cm. longa, 0.5–2 mm. lata; calyce albido-piloso. Type: Baja California, San Gregorio, Feb. 2, 1889, *T. S. Brandegee* [G].)

Material seen—Mexico: Baja California—San Gregorio, Feb. 2, 1889, T. S. Brandegee (C, G Type); Lagoon Head, *Palmer 808* (C, G). The following collections are intermediate between this variety and the var. *deserti:*—Valley of the Colorado, Arizona, *Palmer 388* (C, in part only); Colorado Desert, Apr. 1889, *Orcutt* (M).

29. N. torynophyllum Greenman Zoë 5: 185.   1905; Brand l. c. 152. Pl. 27, fig. 13 and 22.

A prostrate, matted, very leafy, densely cinereous-villous-hirtellous annual, the branches 3–7 cm. long, forming thick leafy mats; leaves spatulate, ca. 1 cm. long, 1.5–4 mm. broad, cinereous-hirtellous above and below, glandular below, revolute, the petioles about as long as blade; flowers many, scattered singly along the branches on slender pedicels ca. 3 mm. long; calyx-lobes linear-spatulate, ca. 3.5 mm. long; corolla tubular, 3–4 mm. long; stamens scarcely extending midway on corolla, unequally inserted about 0.5 mm. from corolla-base, filaments filiform, less than 1 mm. long, the adnate bases neither enlarged nor winged; styles scarcely 1 mm. long; capsules 50–90-seeded, glabrous; seeds brown, ovoid, ca. 0.4 mm. long, shallowly alveolate-pitted and minutely transversely corrugated.

Material seen—Mexico: Peña, Coahuila, *Purpus 124,* Type collection (C, G, M, P, US).

30. N. pusillum Lemmon ex Gray, Proc. Am. Acad. 20: 305.   1885; Brand, l. c. 159.   *Conanthus pusillus* Lemmon ex Heller, Cat. N. Am. Pl. 6. 1798.   Pl. 27, fig. 8 and 26.

A dichotomously branched, densely short-hirsute, diffuse or partially erect annual, forming dense mats 3–20 cm. in diameter, branches often somewhat reddish; leaves rhombic-ovate to obovate, grayish-green due to dense pubescence, the blade 3–6 mm. long, 1.5–5 mm. broad, tapering gradually to a petiole nearly as long as blade; flowers axillary, sessile or nearly so; calyx densely gray-hirsute, the lobes linear or narrowly linear-spatulate, 3.5–4.5 mm. long; corolla pale whitish-pink, narrowly tubular, 4–5 mm. long; stamens unequally inserted 1–1.5 mm. from base of corolla, the free portion ca. 1 mm. long, filiform, the adnate bases bifid and ending in 2 slightly divergent, minute scales that extend nearly to base of corolla; styles 1–1.5 mm. long; capsule ca. 3 mm. long, 20–40-seeded; mature seeds ca. 0.4 mm. long, irregularly-shaped, dark brown, areolate.

Representative material—United States: California—Inyo Co., Emigrant Canyon, *Parish 10192* (G, S); Death Valley, March 12, 1924, *Jones* (P); Panamint Canyon, May 3, 1897, *Jones* (P); San Bernardino Co., Mohave Desert, *Parish and Parish 1329* (G, but not S); near Watermaine, Mohave Valley, *Lemmon and Lemmon 3137* (C, probably part of Type collection); Calico, *Parish 9809* (G, S), *Lemmon 3137* (G, Type), *Lemmon and Lemmon* in May, 1884 (C, US, possibly part of Type collection); Saratoga Spgs., *Muns and Hitchcock 10966* (P); 10 mi. n.w. of Riggs, *Muns and Hitchcock 10949* (P); 37 mi. n. of Baker, *Howell 3601* (CA); Ludlow, *Hall 6110* (C); Kramer, *K. Brandegee* (C); Needles, May 3, 1904, *Jones* (P); near Mule Springs, Riverside Co., *Hall 5963* (C).

31. N. DEPRESSUM Lemmon ex Gray, Proc. Am. Acad. 20: 304. 1885; Brand, l. c. 160. *Conanthus depressus* Lemmon ex Heller, l. c. Pl. 27, fig. 5 and 31.

A dichotomously branched, strigose-hirtellous, diffuse or partially erect annual, the branches 5–10 cm. long, leafy chiefly at the extremities; leaves linear-spatulate to narrowly oblong-spatulate, 10–30 mm. long, 1.5–4 mm. broad, pale green, densely and finely strigose; flowers axillary, clustered near the ends of the branches, sessile or nearly so; calyx-lobes linear, 2.5–3 mm. long, short hirsute-strigose; corolla tubular, ca. 4 mm. long, whitish; stamens inserted ca. 1.5 mm. from base of corolla-tube, filaments filiform, ca. 1.2 mm. long, the adnate portion but slightly expanded and the margins but very slightly free, extending to base of corolla; styles 1–1.5 mm. long; capsules ca. 3 mm. long, corrugated due to pressure of the 16–30 seeds; mature seeds dark brown, ca. 0.6 mm. long, rhomboid-ovoid, with about 20 irregular pits.

Representative material—UNITED STATES: California—without locality, Mohave Desert, May, 1882, *Parish and Parish 1329* (S, but not G); Inyo Co., near Independence, *Hall and Chandler 7293* (C); San Bernardino Co., Newberry, *Munz, Harwood and Johnston 4087* (P); near Calico, Mohave Valley, *Lemmon and Lemmon 3136*, TYPE collection (C, G), and in May, 1884, *Lemmon and Lemmon* (C, US); Daggett, *Hall 6146* (C); Camp Cady, *Parish and Parish 1331* (G); Barstow, *Parish 9218* (S); Rabbitt Spgs., *Parish 9829* (S); Amboy, *Curran* in 1884 (C); Kelso, May 2, 1906, *Jones* (P); Kern Co.—Kernville, *T. S. Brandegee* in 1891 (C).

32. N. EHRENBERGII Brand, Fedde Rep. Spec. Nov. 18: 309. 1922.

Annual, sparingly hirsute; branches ascending, ca. 15 cm. tall; leaves short-petiolate, obovate, ca. 15 cm. (should be "mm."?) long, ca. 5 mm. broad, obtuse, narrowed at base; flowers axillary, short pedicellate, solitary; sepals linear, 6 mm. long in anthesis, enlarging to as much as 9 mm. long; corolla broadly tubular, 8 mm. long; stamens subequal, somewhat dilated at base, free portion much longer than adnate portion; styles connate to middle, shorter than calyx and ovary; ovules about 25 at each placenta; capsule oblong, 2/3 as long as fruiting calyx.

Material seen—None. Above description compiled. The type is a plant collected at San Sebastian, Mexico, April, 1837, *Ehrenberg 960.*

Judging solely from Brand's description, it seems certain that this is a valid species. The connate styles are found in but a few species and *N. Ehrenbergii* is apparently greatly different from any of these.

DOUBTFUL OR EXCLUDED SPECIES

1. *Nama affinis* O. Ktze. Rev. Gen. Pl. 2: 435. 1891 = Hydrolea affinis A. Gray, Man. Bot. No. U. S. ed. V, 370. 1876.

2. *N. caroliniana* O. Ktze. l. c. = H. caroliniana Michx., Fl. Bor. Am. 1: 177. 1803.

3. *N. coldenioides* Jones, Contr. West. Bot. 12: 57. 1908 = Coldenia plicata (Torr.) Cov. The type, *Jones 3869*, from The Needles is certainly not a *Nama.*

4. *N. corymbosa* O. Ktze. l. c. = H. corymbosa Ell. Sketch 1: 336, 1821.

5. *N. cubana* P. Wils. = Hydrolea sp.

6. *N. convolvuloides* and *N. evolvuloides* published by Choisy, DC. Prodr. 10: 183. 1846, in synonymy under *Evolvulus alsinoides*.

7. *N. extraaxillaris* O. Ktze. l. c. = H. extra-axillaris Morren, Ann. Soc. Bot. Gand. 2: 321. 1846.

8. *N. glandulosa* Peter, E. and P. Pflanzenf. 4[3a]: 69. 1893 = Namation glandulosum Brand, Fedde Rep. Sp. Nov. 10: 280. 1912.

9. *N. longifolia* Rusby, Mem. N. Y. Bot. Gard. 7: 337. 1927 = Hydrolea sp.

10. *N. megapotamica* O. Ktze. l. c. = H. megapotamica Spreng. Syst. 4, Cur. Post. 114. 1827.

11. *N. multiflora* O. Ktze. l. c. = H. multiflora Choisy, l. c. 181.

12. *N. nigricaulis* O. Ktze. l. c. = H. nigricaulis Wright, Griseb. Cat. Pl. Cub. 207. 1866.   . .

13. *N. ovata* Britt. = H. ovata Nutt. Trans. Am. Phil. Soc. N. S. 5: 196. 1837.

14. *N. ovata* Harper, not Britton = H. ovata var. georgiana Brand.

15. *N. paludosa* O. Ktze. l. c. = H. paludosa Benn. Journ. Linn. Soc. 11: 270. 1871.

16. *N. Parryi* A. Gray = Eriodictyon Parryi Greene, Bull. Cal. Acad. 1: 202. 1885.

17. *N. quadrivalvis* O. Ktze. l. c. = H. quadrivalvis Walt. Fl. Carol. 1: 109. 1788.

18. *N. racemosa* Kellogg = Phacelia racemosa Heller, fide Curran, Bull. Cal. Acad. 1: 143. 1885.

19. *N. spinosa* O. Ktze. l. c. = H. spinosa L. Sp. Pl. 328. 1753.

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THE AQUATIC FLOWERS OF A TERRESTRIAL PLANT, *HELICONIA BIHAI* L.

Alexander F. Skutch

The profusion of the huge, undivided leaves of the great herbs of the genera *Heliconia* and *Calathea* constitutes one of the most striking and characteristic features of the vegetation of the humid lowlands of Central America. While scattered plants are sometimes encountered in the forest, the largest species of these genera are preëminently plants of open, sunny places. They thrive along the low banks of rivers and lagoons, in abandoned clearings of all sorts, in scrubby pastures, and some kinds in low, swampy places. It is only the smaller-leafed species that are really at home in the original forest. The foliage of some species, especially the great, oblong leaves of *Calathea lutea, C. magnifica, C. insignis,* and of *Heliconia mariae,* stands erect and stiff, and is more bizarre than handsome; but the long and comparatively slender, gently bending, light-green leaves of *Heliconia bihai* are outstanding in grace and beauty. The lamina of flowering plants of this species reaches 3 m. in length, with a width of about 30 cm. This plant occurs throughout the Caribbean lowlands of Central America, but is rather local in its distribution. In some districts it is exceedingly common, while in other intermediate regions, apparently suited to its habits, it is rather rare. Nowhere have I found it more abundant than in the province of Bocas del Toro in western Panama. By virtue of its creeping rhizomes it forms dense clumps in abandoned fields, bushy hedgerows, and along the banks of lagoons. The distinctive form and color of its leaves at once distinguish it from the other great-leaved species with which it grows.

The inflorescence of *Heliconia bihai* is to my mind the most beautiful of all its genus—at least among the Central American members, which alone I know at first hand. It stands in the midst of the leaves, at the top of the short false-stem composed of their overlapping, sheathing bases, usually from 1 to 1.5 m. above the ground (fig. 5). The strongly folded bracts of the flattened inflorescence stand in two ranks, which alternate along the length of the strong, flexuous rhachis. These bracts are highly and attractively colored, and form the chief beauty of the plant. There are from five to ten on each side, according to the size of the inflorescence. The lower bracts are about 20 cm. long, whence there is a gradual reduction in length upward to the most apical, which measures about 10 cm. Sometimes the basal bract bears a reduced but perfect green lamina at its apex, and then it is of course much longer. The central region of each outer face of the folded bract is colored with a bright but delicate shade of red, which pales outwardly to light orange

or sometimes white. This in turn is narrowly bordered with green along the margin and keel of the bract. As the bract ages, the green spreads and encroaches on the brighter color of the center.

The bract is thick and fleshy, and its central portion is occupied by a series of large lacunae similar to those found in the sheath and midrib of the foliage leaf. The free edges closely clasp the rhachis and the swollen base of the next bract above on the opposite side, forming a tight container in which rain water accumulates and remains. During the period of bloom, which on the Caribbean coast of Panama begins with the onset of the drier season in late December or early in January and continues until June, they are found to be constantly filled with water. The inflorescence of all healthy plants is strictly erect, and if for any reason the stem of the plant leans to one side or another, a geotropic curvature of the axis below the lowest bract restores it to the vertical position essential to the holding of its water supply.

The flowers are borne in two ranks along the upper side of the fleshy, somewhat conical peduncle which stands in the axil of each bract, except occasionally the lowest (fig. 1). Each flower is subtended by a large, white,
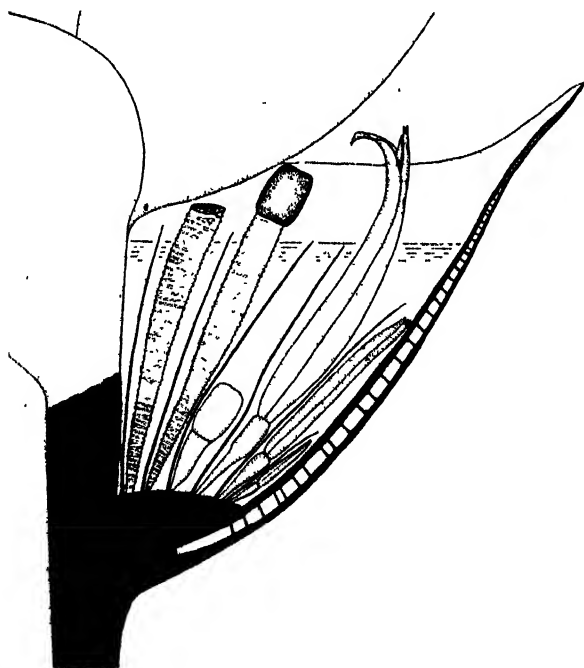
Fig. 1. Portion of the inflorescence of *H. bihai*. A side of a single bract has been cut away to expose the buds, flowers and fruits. There may be seen within this bract, from right to left, two unopened buds, a flower in anthesis, an immature fruit on a short pedicel, a mature fruit on an elongated pedicel, and an elongated pedicel from which the fruit has fallen. Each of these structures is subtended by a bractlet. The level of the water within the bract is indicated by broken lines. Each bract subtends a raceme containing many more flowers than are shown here. March 25, 1929. × 2/3.

chartaceous, ovate-deltoid bractlet, 5 to 5.5 cm. long by 2 to 3.5 cm. broad. Those of the two ranks alternate and somewhat overlap each other. The thick, clavate pedicel of the flower and immature fruit is 1 to 1.5 cm. long. The perianth tube is narrow and contracts upward, 6 to 6.5 cm. long in an-thesis, in color white tipped with green. Five of the six divisions of the perianth are fused into a unit whose compound nature is indicated by the five short teeth (three interior and two exterior) at its summit. The narrow free sepal lies on the abaxial side of the flower and is free almost to its base. It fits so closely around the remaining portion of the perianth that a water-tight seal is formed between the two divisions. The flower bud, enveloped by its bractlet, completes its development wholly submerged in the water which fills the concavity of the bract. The close union of its perianth parts effectively prevents the access of water to its interior. During the night before the flower opens, the tube elongates until its apex extends about 2 cm. above the level of the water, and its tip is just visible above the bract's upper margin. The filaments of the five fertile stamens are so long that the anthers are borne at the upper extremity of the tube, where they surround the capitate stigma, which terminates an equally long style. The pollen, consisting of large, perfectly smooth grains 90 to 100 μ in diameter, and intermixed with numerous acicular crystals derived from the anthers, is shed directly upon the stigma. This occurs early in the morning, as the flower opens by the bending back of the apex of the free sepal. Thus admittance to the long perianth tube is gained through a narrow aperture at its upper end. The pollen grains burst if they are touched by water, and accordingly the complete exclusion of this fluid from the developing bud is of considerable importance to the plant.

At the base of the tube of newly opened flowers is a rich accumulation of nectar secreted through three small pores at the base of the style, which are the orifices of a large, three-armed nectary situated in the axis and septa of the three-celled, three-ovuled ovary.

I have seen the flowers visited only by several kinds of humming-birds. The large, brown Hermit (*Phœthornis longirostris*), with its long, slender, curved bill, is a constant patron of the *Heliconia* flowers. I have frequently watched these humming-birds as, poised on vibrant wings, they sucked nectar from the flowers, and Carriker (1910) records that in Costa Rica this species " is fond of flowers of the wild plantain and feeds on nothing else while they are in bloom." Wild plantain is a term applied indifferently to the various species of *Heliconia* encountered in Central America. In probing the flowers, the bird hovers in a very upright position and bends its head sharply downward to insert its bill into the tube. I have also many times watched the green, ruddy-tailed Rieffer's Humming-bird (*Amazilia tzacatl tzacatl*), the commonest humming-bird in clearings throughout the Caribbean lowlands of Central America, hovering beside these flowers, often in the evening, after they had long been self-pollinated. The bill of this species, only 2.1 cm.

long, is quite inadequate to reach the store of nectar, but the tubular tongue can be extruded for a great distance and probably suffices to attain the desired prize; yet, since the whole bird measures only 9.8 cm. in length, it must require quite a stretching, if indeed it is actually able to do so. In addition to these two, I have seen a third, unidentified humming-bird, a brown Hermit of medium size, poised before the flowers.

It has already been mentioned that the pollen is shed directly upon the stigma at the time the flower opens. In view of this circumstance the significance of the visits of the humming-birds is not easy to determine. Possibly the pollen brought on the bill of the bird from another plant is prepotent over the flower's own pollen, but in the absence of this the flowers are self-fertilized. In the lowlands of Guatemala, where the species is abundant, I bagged two developing inflorescences, just on the point of opening, on February 28. By the end of April no seeds had matured, but on May 15 I collected 109 seeds from the two inflorescences, and on June 11 an additional 49. More detailed studies of ornithophilous flowers are certainly much to be desired, especially since doubt has been cast upon the utility of the visits of birds to *Marcgravia,* Thomas Belt's classic example of ornithophily, by the discovery of Bailey (1922) that two species at least (*M. cuyuniensis* and *M. purpurea*) are, like *Heliconia bihai,* self-pollinated.

The style and filaments of *Heliconia bihai* continue to elongate after the pollen has been shed. These slender organs thereby become much crumpled in the tube, and finally often force the anthers and stigma to emerge from its tip, but by the time they become exserted they are almost always discolored and in early stages of decay.

The flower parts above the inferior ovary decay beneath the bractlets without abscission. Their decomposition, together with that of various kinds of foreign matter which collect in the little pools of water in bracts, causes them to become disagreeably foul. They swarm with aquatic life, conspicuous among which are the large " rat-tailed larvae " of a drone fly (*Eristalis* sp.) and numerous mosquito " wrigglers " (*Culex bihaicolus* D. & K.).[1] The former are of a genus well-known for its preference of foul water in which to deposit its eggs; and these particular larvae are able to crawl over the surface of the plant to a pool inside another bract in case their native pond is drained, which happens when the Purple Gallinule tears apart the bracts to obtain the seeds. Doubtless the protozoologist would find these little aerial pools a rich collecting ground.

The fruit develops in complete submergence. While unripe, its surface is pure white; but upon the approach of maturity it takes on a lavender tint which finally deepens into a rich cobalt blue. In shape the berry is oblong, 11 mm. long by 9 mm. in diameter; and it contains at most three elongate seeds, 11 mm. in length, with a warty, brown, horny seed coat. The thin

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[1] For the determination of these larvae I am indebted to Dr. C. T. Greene of the National Museum.

flesh of the fruit is almost tasteless, but the presence of numbers of free raphid cells containing bundles of acicular crystals causes a stinging sensation in the mouth, somewhat milder than that produced by the corm of the Jack-in-the-pulpit.   It contains no starch, but is rich in oil globules and is eagerly sought by birds.

At maturity the fruit is pushed above the surface of the water by the rapid elongation of the pedicel, which, because of its interesting behavior, deserves special attention.   The numerous vascular bundles are closely aggregated at the center of the thick, club-shaped structure characteristic of the unripe fruit (fig. 2).   The outermost of these bundles are accompanied by strands
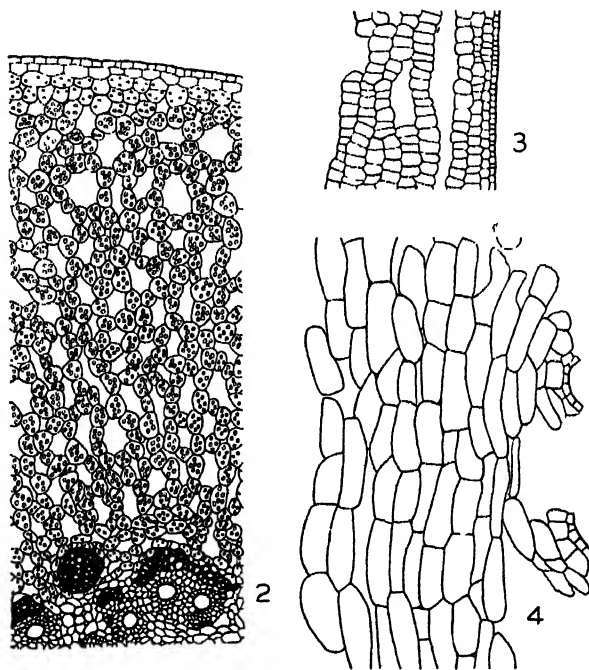
Fig. 2-4. Fig. 2. Portion of the cross-section of the unelongated pedicel of a full-grown, almost mature fruit, showing the central cylinder with its vascular bundles and fibrous strands, and the cortex with its large lacunae and thin-walled, starch-filled cells. Fig. 3. A portion of the cortex and epidermis of a similar pedicel in longitudinal section (starch grains not shown). Fig. 4. A corresponding section of a pedicel after complete elongation, showing the rupture of the epidermis and the great increase in length of the cortical cells. All figures from camera lucida drawings, × 50.

of thick-walled, lignified fibers. This central cylinder of the pedicel is surrounded by a lacunar tissue composed of short, rounded cells enclosing large air canals—a typical cortex for a submerged organ! The walls of these cells are very thin, and their cavities are filled with numerous, large starch grains. After the berry has ripened, the pedicel begins to elongate, increasing in a short time from 1 or 1.5 cm. to between 4.5 and 6 cm. in length. The elonga-

tion is effected entirely by the growth of the cells of the cortex. The central strand, with its thick-walled fibers, is incapable of stretching and pulls away from the surrounding cells. Thus the extended pedicel becomes a tube in all except its basal portion. The epidermal cells do not elongate, and the two or three subjacent layers of angular cortical cells increase very slightly in length. They are torn by the growth of the pedicel into a number of narrow, transverse rings, which continue to surround the organ and give its surface a corrugated appearance (fig. 1, 4). Those regions which do not elongate are poor or lacking in starch, in sharp contrast to the lacunar tissue.

The elongation of the pedicel is effected merely by the stretching of the walls of the cortex, unaccompanied by cell division. In the pedicel of the immature fruit, these cells measure 19 to 44 μ in length, while upon elongation they attain a length of 96 to 272 μ—an increase of about five or six times, or roughly in the same ratio as the change in length of the entire pedicel. The starch completely disappears from these cells during the process of elongation. Although no chemical tests could be made, the starch is probably converted into sugar, which increases the osmotic pressure of the cortical cells, or is otherwise used in performing the work of growth. Only the pedicels which bear fertile fruits ever elongate. This rapid stretching of the spongy tissue reminds me more of the sudden elongation of the stipe of the fungus *Mitremyces* than any kindred phenomenon among vascular plants with which I am personally familiar.

By the elongation of the pedicels the fruits are raised above the bractlets and reach the level of the edge of the bract, where their glossy, intensely blue surfaces catch the eye of passing birds. Despite their unpalatability to the human taste, they are eagerly sought by birds of several species. The Purple Gallinule (*Ionornis martinica* L.) is the bird I have most often observed eating them. I know of no more attractive picture of tropical wild life than that formed by the lovely creature as it clasps the gayly colored bracts with its long, yellow toes and pecks at them with its bright red bill. Not content to wait until the normal elongation of the pedicel brings the ripe fruit within easy reach, the bird tears apart the thick bracts to obtain the ripening but still submerged berries. The inflorescences are greatly mutilated by this activity, and great numbers of flowers and torn bractlets strew the ground beneath them (fig. 6). Perhaps, too, the bird consumes some of the larvae which the bracts harbor. The plant pays dearly in destroyed and blasted flowers and young fruits for the dispersal of its seeds by the Purple Gallinule. The Scarlet-rumped Tanager (*Ramphocelus passerinii*) also visits the inflorescences and probably feeds on the berries.

In the valley of the Rio Morjá on the boundary between Guatemala and Honduras, I frequently found the bracts of this plant torn to pieces, in much the same manner as I had seen them mutilated in Panama three years earlier. The Purple Gallinule, which despite its vast range is a bird of very local occurrence, was not met during five months of field work on the plantation

where these observations were made, and I was never fortunate enough to discover the creature responsible for these depredations on the plant. I rather suspect the White-bellied Wood Rail (*Aramides albiventris*), which is quite abundant in the region, but the matter still remains a mystery to me.

Although *Heliconia bihai* is decidedly a terrestrial plant, its flowers and fruits develop in an aquatic medium, in little private pools which collect in the concavity of each cupped bract. One at once seeks resemblances to the flowers of truly aquatic plants. In many of these, as in *Heliconia*, the buds

Fig. 5–7. Fig. 5. A group of inflorescences of *Heliconia bihai* L. Almirante, Panama, January 8, 1929. Fig. 6. An old inflorescence of *H. bihai,* showing the work of the Purple Gallinule in tearing apart the bracts. April 27. 1929. Fig. 7. The pendent inflorescence of *Heliconia mariae.* Almirante, Panama, January 8, 1929.

develop in complete submergence, and water is excluded from their interior by the close-fitting perianth parts. Except in certain sections of the family Potamogetonaceae, the pollination of the flowers of most aquatic plants occurs above the surface of the water. One is reminded of the long perianth tube of the pistillate flower of *Elodea,* which bears the stigma just above the surface of the pond, while the ovary remains submerged. It is a peculiarity of the flower of *Heliconia* that, although its pollination is subaerial, the birds which visit it must stick their tongues well below the surface of the water to reach the nectar which attracts them. In the ripening of its fruits in complete submergence, *Heliconia* agrees with a large number of aquatic plants

which, like it, are specialized for subaerial pollination; it is necessary to mention only *Elodea, Valisneria, Peltandra,* and *Heteranthera,* among many others.

The fact that the flowers of a species of *Heliconia* (called *bihai* but judging from the illustration apparently not this species) develop beneath the water which collects in the bracts was observed by Schimper (1903), who describes also the similar situation which prevails in *Nidularium* and certain other bromeliads, where the entire inflorescences develop submerged in the water which collects between the leaves of these tank epiphytes. In *Mendozia velloziana,* one of the Acanthaceae, the fused bracts form water-filled cups in which the flower buds develop. A phenomenon somewhat kindred to these is found in the so-called water-buds of a number of tropical plants, of which *Spathodea,* described by Treub (1890), is perhaps the classic example. But the situation in these is really quite different, since the flower buds of these species develop in the air, although their calyces are turgid with water secreted into them by internal glands, and bathed in this the corolla, stamens, and pistils develop. In *Heliconia bihai* and a few related species the water is held outside instead of inside the calyx. In either case it may prevent the access of destructive insects or their larvae to the essential organs, if this is really the function of these peculiar arrangements.

What advantage this aquatic habit of its flowers may be to *Heliconia bihai* is a matter of pure conjecture. Many related species, as *H. mariae,* produce pendent inflorescences in which, of course, standing water cannot collect. The spaces beneath the bracts of these plants, as is the case also with the close-bracted inflorescences of many species of *Calathea, Costus, Renealmia,* and other Scitamineae, often swarm with ants, cockroaches, and other insects which, added to the slime and the decaying vegetable matter which also are found there, make them very unpleasant to handle. The creatures which they harbor doubtless inflict a certain amount of injury to the flowers; yet these do succeed in setting a sufficient number of viable seeds to propagate the species. Ants and most other mature insects are held aloof from the developing flower buds, the ovaries, and the immature fruits of *Heliconia bihai* by the aquatic medium with which they are bathed, and the larvae which develop there apparently do not harm them.

A comparison of the inflorescence of *Heliconia bihai* with that of *H. mariae* Hooker reveals certain interesting peculiarities in the former which appear to be correlated with the aquatic environment of its flowers. *H. mariae* is the largest and most abundant representative of its genus in Bocas del Toro province, and is widespread throughout the Central American lowlands. It grows on rather dry hillsides, well-drained banks of lagoons, and in second-growth thickets in level areas, provided the ground is not marshy. Plants favorably situated attain a total height of 9 m., and produce heavy, pendent, flattened inflorescences which sometimes reach a length of 120 cm. (fig. 7). The closely overlapping, two-ranked bracts are of a lurid, dull-red

color which earns for the species the appropriate, if unflattering, name " Beef-steak Heliconia."   The flowers are essentially similar in structure to those of *H. bihai,* but the perianth tube is shorter, in anthesis only 4 cm. long, white at the base and deepening to red at the apex.   They develop beneath closely overlapping bractlets which resemble those of its congener.   In anthesis the tip of the flower protrudes about a centimeter beyond the bracts.   The blossoms are much visited by humming-birds of several species; but, as with *H. bihai,* the stigma, at the beginning of anthesis in the early morning, is found to be laden with pollen from the anthers which surround it in the tip of the perianth tube.   I have seen eel-worms writhing in the pollen mass!   The bractlets gradually decay after the anthesis of the flower they subtend, and there is no proper abscission of the perianths, which rot away between the bracts, adding to the foulness which prevails there.   The decay of these tissues is hastened by the moisture which collects abundantly among the closely packed organs, although the pendent position of the inflorescence prevents the accumulation of standing water.   The entire arrangement seems a great and foul extravagance of a riotous tropical nature, and is as forbidding as it is bizarre.

One of the marked differences between the two species is found in the structure of their floral pedicels.   In anthesis, that of *H. mariae* is only slightly the shorter, and is about 1.1 cm. long.   The vascular bundles are collected in its center, but are more scattered than in *H. bihai* and devoid of heavily thickened mechanical elements.   The cortex contains air spaces, but these are fewer and smaller than in the submerged pedicels of the other.   Its component cells, as well as those in the ground tissue between the vascular bundles, are collenchymatously thickened, while those of *bihai* are thin-walled. There is a small amount of starch in the cells surrounding the vascular bundles in the pedicels of ripening fruits, and scattered small grains occur in the inner part of the cortical tissue, but the total amount is very slight when compared with that of its congener at a corresponding stage.   Both the central region, with the vascular bundles, and the epidermis elongate harmoniously with the remainder of the pedicel, which accordingly remains solid and continues to be covered with the epidermis to the end.   The total elongation is less than that of the pedicel of *bihai,* and a final length of 3 to 4 (rarely 5) cm. is sufficient to expose the cobalt-blue fruit beyond the bract.   It is interesting that in this species the pedicel elongates, whether or not it supports a seed-bearing fruit, and the process of elongation is much more gradual.   The berries are so similar in appearance and taste to those of *H. bihai* that doubtless they are equally acceptable to birds, although I do not recall actually having seen them eaten. Sometimes the seeds are viviparous and germinate while still held between the bracts.   In my experience it is somewhat easier to obtain good seeds of *bihai* than of *mariae;* yet on any basis of comparison the number of seeds produced by a *Heliconia,* in return for a tremendous expenditure of material in creating the ponderous inflorescence, would seem immoderately extravagant in a plant of the temperate zones.

SUMMARY

The fleshy bracts of the erect inflorescence of *Heliconia bihai* form cups in which water collects and remains during the period of flowering. The flower buds develop in complete submergence in these little aerial pools, while the close-fitting parts of the perianth prevent access of water to the interior. At the time of flowering the perianth tube elongates until its apex, in which the stigma and anthers are borne, protrudes above the surface of the pool. The flowers are visited by humming-birds; yet at the beginning of anthesis the pollen is shed directly upon the stigma, and seeds are set freely in bagged inflorescences. The fruit develops completely submerged in the pools, but upon maturity is carried above the level of the water by the rapid elongation of the pedicel. The epidermis and central cylinder do not participate in this elongation, which is effected by the stretching, without division, of the cells of the lacunar cortex. The bright-blue berries are sought by several kinds of birds, which often inflict great injury to the inflorescence in their eagerness to reach them before they are duly exposed by the elongation of the pedicel. A comparison of *H. bihai* with *H. mariae*, a species with a pendent inflorescence, indicates several interesting modifications in the structure of the pedicels of the former, apparently associated with their aquatic environment.

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A CASE OF EPHARMONY IN A NEW ZEALAND *RUBUS*

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Under the term " epharmony " are here included all morphological changes that can reasonably be considered as due to a change of environment, and the plant so changed may be conveniently called an " epharmone." Evidently epharmony is the concern of taxonomy as well as of general biology. " Epharmone " is an unambiguous name for those distinct modifications of form met with in nature but usually either neglected in floras or classed as " varieties "—a term so frequently used as a dumping-ground for forms of more than one biological status. In ecology too, epharmones of different life-forms from the so-called " normal "—" usual " may be a better designation—play quite different parts in the vegetation, and to apply to them merely the specific name is misleading in the extreme. The case here presented may serve, in part, to illustrate these remarks.

In New Zealand, as elsewhere, the genus *Rubus* exhibits polymorphy to an astonishing degree. Yet the work of the last fifteen years has brought all local botanists to believe that the diversity of form in species generally is principally due to hybridism and epharmony. As the species now recognized number five only, and as their limits are already pretty well known, they give a convenient starting point for the task of bringing the forms into definite taxonomic groups. If this work be based on the recognition of the true-breeding units—the " jordanon " in my sense of that term—together with adequate study of epharmony and hybridism in the field, it is by no means so hopeless as might appear from the bewildering array of forms seen in herbaria, collected without reference to ecological evidence. Such work when completed will be based not on botanical opinion but on actual fact.

Except for a creeping and rooting mat-plant, the New Zealand species, when fully developed and in their usual habitat, are high-climbing, scrambling lianes. Their main stems are rope-like, usually unbranched, covered with thick bark, and are many meters in length, with a diameter up to 15 cm. or even more. The leaf-stalks, and usually the midribs, are furnished abundantly with hooked prickles and serve as efficient climbing organs. Thus armed, the plants seize tenaciously anyone coming in contact with them, so that the vernacular name in New Zealand for *Rubus* is " Lawyer."

In the species under consideration, *R. cissoides*,[1] the leaves are for the

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[1] This is the name applied to the species by all local botanists, but Dr. H. H. Allan, who has examined the type material at the herbarium of the Royal Botanic Gardens, Kew, considers that *R. cissoides* A. Cunn. was based on a North Island jordanon of the compound species known to local botanists as *R. australis.*

most part trifoliolate, and show an extreme diversity of lamina development. At one extreme are the "midrib leaves" (made up really of petiolules, as shown by Goebel, but looking so much like midribs that for the sake of emphasis I so designate them); at the other the fully developed "lamina leaves." In the midrib leaves the laminae are tiny, and sometimes reduced almost to the vanishing point. They are situated at the tips of the elongated leaf-stalks, which are strongly armed with yellow prickles. In the lamina-leaves the blades are well developed, and the stalks shorter, with fewer, weaker prickles. Between the two extremes a whole range of intermediate forms may be observed (fig. 1). That the term "midrib leaf" is not inappropriate may be seen by a study of the leaf-development from the seedling stage of this, as of the other New Zealand compound-leaved species of *Rubus*. Commencing with simple leaves, there is a transition by way of lobed and partite to the fully trifoliolate, with the occasional production of still further leaflets. In the adults, too, one often finds a parallel series of forms, though

TABLE 1. *Differentiae for the two extreme epharmones of Rubus cissoides*

	Liane-form epharmone R. cissoides eph.* foliosus	Bolster-form epharmone R. cissoides eph.* pauperatus
Life-form	High-climbing, scrambling liane	Bolster-like plant, of densely entangled stems forming mounded elastic masses
Main-stems	Long, rope-like, swinging free from supporting plant	Shorter, soon branched, producing a dense bush
Leaves	With well-developed laminae	With greatly reduced laminae
Petioles	$\pm$ 4 cm. long, sparingly armed with pale yellow prickles	$\pm$ 8 cm. long, more strongly armed with bright yellow prickles
Petiolules	$\pm$ 3 cm., sparingly armed with prickles, or quite unarmed	$\pm$ 15 cm., strongly armed with prickles
Laminae	$\pm$ 5 cm. long by $\pm$ 1.75 cm. broad, rather distantly and coarsely serrate, midribs occasionally bearing one or two weak prickles	$\pm$ 1 cm. long by 2 mm. broad, or still further reduced, sparingly toothed or almost entire, often lobed at base
Flowers	In well-developed panicles	Not produced

* Eph. = epharmone and is here used instead of var., which I consider should only be used for a true-breeding group (jordanon).

these are not produced in any regular order (fig. 2). Entire plants may show nothing but the midrib form, but the plants growing as lianes on the forest margin more often show both forms, along with intermediates. Though it is easy to observe that the two classes of leaves are directly a matter of epharmony, the form with midrib leaves only is treated in floras as a taxonomic variety under the name "*pauperatus*." The production or

Fig. 1 (above). *R. cissoides*. Leaves taken from a flowering plant, showing range of form from lamina to midrib leaves. Fig. 2 (below). *R. cissoides*. Leaves from adult liane, showing transitions from unifoliolate to tri- and pentafoliolate forms.

the suppression of laminae is, however, due to the degree of illumination to which the plant, or a part of it, is exposed, and to its hereditary constitution. Where the forest canopy is comparatively dense, the liane has foliage of the lamina form, but where certain parts of the plant are exposed to much brighter light, these show every gradation toward midrib leaves. Where a part is exposed to the full light, it becomes quite of the midrib form. Thus the commonest form of the forest-liane presents a curious blending of the two distinct leaf-types. Yet where the liane is exposed in all parts to full sunshine, it bears purely midrib leaves, and the branches are very densely entangled. Messrs. G. Simpson and J. Scott Thomson, who have carefully examined numerous specimens near Dunedin in South Island, report an example of this nature completely investing a small tree with a dense mat 5 m. tall and over 1 m. in diameter. Their observations, however, on plants in modified forest (more sunny and with drier atmosphere than in primeval forest) lead them to the conclusion that midrib leaves on young plants in the forest are attuned to more shade than the lamina-leaves of the adult. Certainly the lamina-leaves, when once developed, are able to tolerate a good deal of exposure to sun and wind. It is also clear that the liane-habit is not in itself a prime cause of the production of lamina-leaves.

In open country *R. cissoides* is also frequent in many parts of the main islands of New Zealand, where it is met with in dry, sunny situations, especially the faces of river-terraces and small screes. In such places bolster-like shrubs made up of densely interlaced twigs are of physiognomic importance. These are found in the Ranunculaceae (*Clematis*), Polygonaceae (*Muehlenbeckia*), and Rosaceae (2 species of *Rubus*). All of these, under forest conditions, are lianes. In its habitat of the open *R. cissoides* is quite a handsome plant, with its wealth of green leaf-stalks furnished with many hooked, bright yellow prickles, and its particularly dense, wind-resisting habit (fig. 3). More remarkable still is the fact that this open-ground epharmone has never been known to flower, though the liane epharmone of the forest flowers and fruits freely on the portions that have leaves of the full lamina form or but slightly reduced. Yet *Rubus subpauperatus* (fig. 4), often a companion plant, and forming similar bolsters in the open, with its leaves also in large measure reduced to the midrib form, flowers freely in this state, as do *Clematis afoliata* (the name most expressive) and the truly leafy *Muehlenbeckia complexa* var. *microphylla*.

At first thought this inability to flower of the open-ground epharmone, and of shoots that bear only midrib leaves, may seem a rather difficult problem to solve, for the plants must reach a considerable age. But the cause was revealed almost by accident, when, some thirty years ago, I was engaged in the study of seedling New Zealand plants, largely with epharmony in mind. I raised seedlings of *R. cissoides* from seed taken from a leafy forest plant. The early leaves were simple, ovate, and deeply toothed, with very short petioles. These were followed by lanceolate leaves of much greater size, and
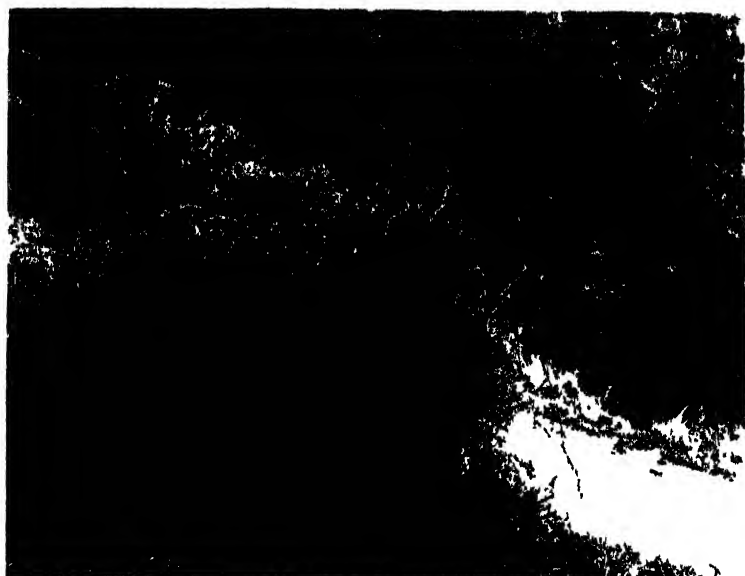
Fig. 3 (above). *R. cissoides*. Bolster-like plant of open situations exposed to much sun and wind. Fig. 4 (below). *R. subpauperatus*. Bolster-like plant of open situations exposed to much sun and wind.

with petioles longer than the laminae.   To my surprise the early juvenile fol-
lowing the seedling was of the midrib form, as seen in figure 5, which is really
the prime motive for this communication.   Thus the bolster form of the open
does not flower because it is a prolonged juvenile form, and such in other
species usually do not produce flowers.[2]   How delicate the balance may be is
illustrated by a plant I observed at the foot of a coastal cliff near Christchurch
in South Island.   Here there was a prolonged juvenile of the usual bolster
form from which, where the cliff gave more shade and shelter, had developed
a long, erect, flowering, leafy shoot.   Dr. Allan, on examining specimens
growing in North Island on a river-terrace, informs me that he noticed a plant
on the upper terrace edge that was of bolster form on the exposed side and of
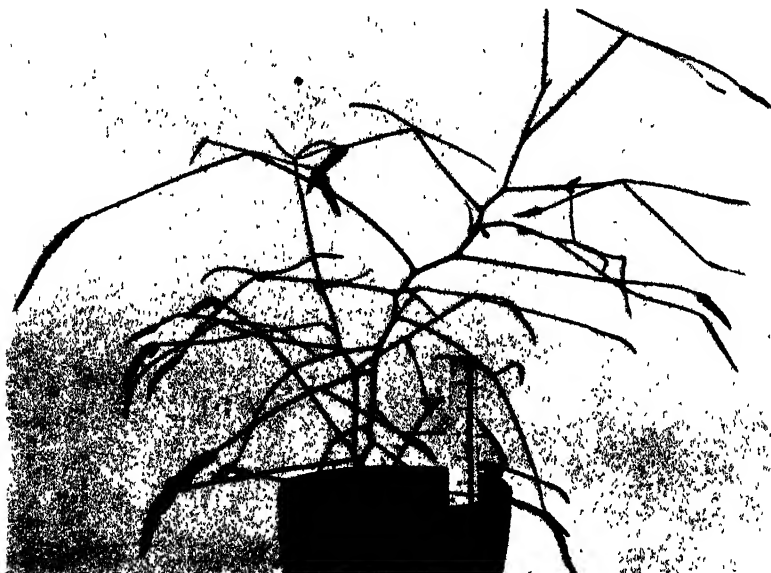
Fig. 5.   *R. cissoides.*   Juvenile plant raised from seed by the author.

leafy habit on the more shaded and sheltered down-terrace side.   This·por-
tion had developed flower-buds.   He also states that several old plants had
produced adventitious shoots of purely midrib form from near the bases of
the main stems, these shoots being in light shade.   This is in accordance with
the phenomena of reversion shoots on the New Zealand heteroblastic species
in general.   Messrs. G. Simpson and J. Scott Thomson report that they con-
sider that *all* spreading growth is at first juvenile in form, and that sunlight
is needed to induce the adult growth.   They view the lamina form as a tree-
top form, but found also in sunlight at forest margins.   They agree, however,

[2] A few cases are known where the juvenile form occasionally flowers—e.g., *Pen-
nantia corymbosa, Podocarpus dacrydioides,* and there are others.

A number of other heteroblastic species may produce reversion-shoots of juvenile
form that flower freely. ·

that the prolonged juvenile midrib form produces bolsters in fully open situations, and that these develop neither lamina leaves nor flowers, though they carry much well " ripened " wood.

SUMMARY

Rubus cissoides is a heteroblastic species with a very distinct juvenile stage, in which the leaves are of midrib form. This juvenile is apparently in tune with forest conditions. Plants growing in the open on dry ground, and exposed to full sunshine, with frequent high winds, produce dense bolster-like masses, and the leaves are all of midrib form. Such plants do not flower, though they attain a large size, and evidently a considerable age. This behavior as to flowering is in great contrast to that of *R. subpauperatus,* which also forms bolsters in the open, but flowers and fruits freely. When growing as a liane of the forest, *R. cissoides* develops to a lamina leaf form, flowering and fruiting abundantly. The balance between the leaf forms is very delicate, and the plant epharmones readily in one or the other direction according to the degree of sunlight and of exposure it, or even a part of it, experiences. Apart from the important biological and ecological problems raised, and so far only in small part solved, the case illustrates the great importance of a full consideration of epharmony in taxonomic studies.

NGAIO, WELLINGTON,
    NEW ZEALAND

THE GENUS *SCHIZOPHYLLUM*. I. SPECIES OF THE WESTERN HEMISPHERE [1]

DAVID H. LINDER

INTRODUCTION

Recently the writer has had occasion to determine a tropical species of *Schizophyllum*, and although the species under investigation possessed microscopical characters that appeared to separate it sharply from what has commonly been accepted as *S. commune* Fr., there was no reference in the literature to such characters. It thus became evident that this genus was in need of a careful revision. The reason for this becomes the more apparent when it is realized that the genus is cosmopolitan in its distribution and therefore grows under extremely variable conditions. Although thirteen species, more or less, have been described, based for the most part on gross morphological characters, but few of them appear to have been thought worthy of recognition. It may possibly be said that most mycologists have concurred with the opinion of the late C. G. Lloyd (1913–1916) when he stated, " . . . , I think there is in reality only one species and but very few collections are entitled to a separate name even as a variety." This view, which is conservative to say the least, is readily explained by the striking variations that result from the interacting influences exerted by such environmental factors as the nature and the position of the substratum, humidity, temperature, and light. In turn, variation in gross morphology frequently obscures characters that are of prime importance, and to judge the value of which a large number of sections must be made of specimens, not only of different ages, but also from different localities. Hence the problem narrows down to a study of the microscopical characters that may aid in separating species.

The present paper is intended to be a survey of those species that occur in the Western Hemisphere. The reason for this limitation is that material of such species is more abundantly represented, but more important still, that either type or authentic material of the majority of the species is available for study in the Farlow Herbarium of Harvard University. It is intended, as material from other regions becomes more ample, and as soon as it is possible to study either type or authentic specimens, to publish on the species from other parts of the world. It may be objected that without a complete knowledge of the European specimens, there is some risk of adding to the confusion in nomenclature. A brief survey of the literature, and of European and oriental material, however, indicates that with the exception of

S. commune and *S. flabellare* Fr., Old World species do not enter into the synonymy of those described from the Western Hemisphere. It is therefore hoped that by giving more complete descriptions and by illustrating the microscopical characters which separate the species, there will result a better understanding of the American members of the genus *Schizophyllum*. As matters stand today, *S. commune,* as it was represented in the Farlow Herbarium, and as it probably still is in many other herbaria, is an accumulation of specimens that includes two or more species and means little more than an assemblage of materials from different parts of the world.

<div align="center">MATERIALS AND METHODS</div>

In making a study of this genus, it was found that the most important characters are microscopical ones. It therefore became necessary to prepare a large number of mounts. The sections, with but few exceptions, were all cut by hand and at right angles to the gills. They were then transferred from 95 per cent alcohol directly into the mounting medium, which was either lactophenol-cotton blue or pure glycerine to which was added a small amount of a saturated solution of eosine in 95 per cent alcohol. The latter medium appears to be the better for permanent mounts, but unfortunately the sections do not recover their turgidity until after a long period of immersion. For this reason a duplicate series of sections was mounted in the lactophenol mixture, and these were employed in the present study. It has been the writer's experience that while lactophenol in many cases is a satisfactory medium for permanent mounts, in some instances, after the preparation has stood for some time, minute drops of an oily substance become so abundant as to obscure minute details of structure and to make the discovery of spores almost an impossibility.

Basidiospores, because of their small size, are rather difficult to use for taxonomic purposes. For this reason the writer has employed a 90 × water immersion objective and a 15 × compensating ocular. With lower magnifications than are obtained with this combination, it is difficult to discern with any degree of certainty the difference between the spores of *S. commune* and *S. radiatum,* for example. While the method may be considered laborious, it has been found that measurements can be more accurately made when the spores are carefully outlined with the aid of a camera lucida. This method has the additional advantage of being an aid in visualizing and comparing the shapes and sizes of a large number of spores.

For making measurements of the fruiting bodies, a set of arbitrary standards was adopted, as explained in figure 1. Thus the thickness of the context (*co*) was measured along line *A,* which extends from the base of the hymenium to the outer limit of the context bordered by the loosely intertwined hyphae of the pellicle (*pel*). The length of the gills was determined from a line projected laterally from the surface of the hymenium to the tip of the gills, an allowance having been made for the curvature of the tip, as shown

by line B. The thickness of the gills was determined, at the base as shown at C, by measuring from the surface of the hymenium to the outer surface of the context but excluding the hyphae of the pellicle. Similarly, the width of the tips of the gills was measured at the point of curvature (D), but in this instance the abhymenial hairs were excluded. While such a standard is subject to a certain amount of variation because of the nature of the object and because of the personal equation, nevertheless it is thought that the system is decidedly better than the use of such ambiguous phrases as "at the base" or "near the tip" without giving them a definite meaning. In all cases, the measurements of the gills were made from the longest pairs, since the tabulation of all variations encountered in a single fruiting body
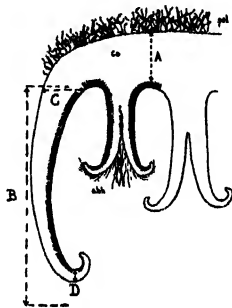
FIG. 1.   Cross section of a fruiting body to show the method of making measurements.

would obscure any real differences in gill length that might and actually do exist.

Before commencing the taxonomic portion of this paper, mention should be made of one more character which if not the most important is at least the most obvious one that can be utilized for the separation of species. This is furnished by the hairs that occur on the opposite side of the gill from the hymenium. These hairs, for want of a better and more concise term, are called "abhymenial hairs." They occur on the terminal portions of all the gills and are quite characteristic for the species. They may be either simple or branched, straight or undulate, and either hyaline or colored. Occasionally they may be roughened with crystalline deposits, but the deposits are not constant in their occurrence and when present they are not specific.

TAXONOMIC CONSIDERATIONS

Schizophyllum Fries, Observ. Myc. 1: 103. 1815; System. Myc. 1: 330. 1821.

Plants leathery, reviving when moistened, solitary, gregarious, or cespitose; pileus covered by a pellicle of loosely interwoven hyphae; the content composed of closely aggregated hyphae with relatively narrow lumens; gills bifurcate and clothed near the extremities with abhymenial hairs, in dry weather mostly inrolled to cover the hymenial layer; stem lateral, eccentric,

or lacking, and when present usually short and always attached to the pileus on the opposite side from the hymenium; spores hyaline, smooth, ellipsoid, or cylindrical; cystidia present or absent. Parasitic or saprophytic, mostly xylophilous.

Type species: *S. commune* Fr.

The genus is unique in that it possesses cleft gills and therefore should not be confused with any other groups in the Agaricaceae. Within the genus there are two easily separated groups which might be entitled to recognition were it not for the few species of which *Schizophyllum* is comprised. The first group, represented by *S. commune, S. radiatum, S. brevilamellatum,* and *S. fasciatum,* is characterized by the context which is composed of densely interwoven hyphae, the lumens of which are approximately one-half the diameter of the filaments (compare fig. 5 and 6 of pl. 36), and also by the thin-walled young basidia. The other group, composed of *S. umbrinum* and *S. Leprieurii,* is recognized by the context that is made up of hyphae of which the lumens are only about one-third the diameter of the filaments, and by the young basidia of which the lateral and terminal walls are conspicuously thickened (fig. 6, 8, 12, pl. 36).

The synonyms of *Schizophyllum* have not been listed, since the majority antedate 1821 and hence have been listed by Fries.

KEY TO THE SPECIES

1. Pileus usually more than 1.5 cm. broad; subhymenium differentiated from the context of the pileus; walls of the young basidia uniformly thin.........................2
1. Pileus usually less than 1.5 cm. broad; subhymenium not differentiated from the context; basidia with much thickened hyaline or lightly colored walls.................5
2. Subulate cystidia absent; abhymenial hairs usually hyaline, simple or furcate near the apex..3
2. Subulate cystidia present; abhymenial hairs brownish, geniculate and irregularly short branched near the apices................................... 4. *S. fasciatum*
3. The length of the mature gills less than the thickness of the context; fruiting bodies thick; spores 6–7.5 × 2.5–3μ............................. 3. *S. brevilamellatum*
3. The length of the mature gills exceeding the thickness of the context; fruiting bodies mostly thin..4
4. Basidiospores cylindrical and obliquely apiculate, 4–7.5 × 1.5–2μ.
　　　　　　　　　　　　　　　　　　　　　　　　　　1. *S. commune*
4. Basidiospores ovoid or elongate-ellipsoid and obliquely apiculate, 4–6 × (1.5)–2–2.5μ
　　　　　　　　　　　　　　　　　　　　　　　　　　2. *S. radiatum*
5. Abhymenial hairs brown, simple, undulate, closely applied to the outer surfaces of the gills.. 5. *S. umbrinum*
5. Abhymenial hairs light brown, branched near the apices, the branches hyaline, coiled or contorted.. 6. *S. Leprieurii*

1. **Schizophyllum commune** Fr., Observ. Myc. 1: 103. 1815; System. Myc. 1: 330. 1821.
　　Agaricus alneus Linnaeus, Sp. Pl. ed. 1, 2: 1176. 1753.
　　Schizophyllum alneum (L.) Schröt., Pilzfl. Schles. 1: 553. 1889.
　　?Schizophyllum Miia Scopoli, Hist. Nat. 4: 147. *pl. 1, fig. 4.* 1769.

Hyponevris alneus (L.) Earle, N. Y. Bot. Gard. Bull. 5: 412. 1909.
Schizophyllum vulgare Lloyd nom. nud., Mycological Writings (Letter
    29) 3: 4. 1909–12.
    Plate 33, fig. 6; Plate 34, fig. 1; Plate 35, fig. 1–6

Pileus thin, coriaceous, suborbicular, flabelliform, or deeply cleft, sessile
or attached by a marginal stipe, 1–5 × 1–6 cm., with a villose pellicle, the hairs
frequently matted, zonate or azonate, grayish-white to somewhat brownish;
context hyaline but frequently brownish discolored in a thin peripheral zone,
the hyphae densely aggregated and with thickened walls, (240)–320–640–
(760)μ thick; the gills longitudinally cleft, radiating from the point of attach-
ment of the pileus and interspersed with shorter ones, buff colored, grayish,
or grayish-brown, hirsute on the abhymenial side, (360)–800–1400–(1700)μ
in length; abhymenial hairs stout, simple, hyaline, thick-walled, and often
granular incrusted; basidia narrowly clavate, 16–29 × 2.5–3μ, with four
slender sterigmata; spores hyaline, cylindrical and obliquely apiculate, 4–7.5 ×
1.5–2μ.

Mostly on wood of deciduous trees, rarely on coniferous wood, and some-
times on roots and stems of herbs. Saprophytic or parasitic.

Rather common in temperate America, but apparently rare in the tropics.

Since Fries' "Systema Mycologica" has been accepted as the point of
departure for this group, there appears to be no reason why the earlier
Agaricus alneus L. should be resurrected to replace the widely accepted name
of Fries. *S. Miia* as depicted in the original figure by Scopoli is apparently
a sessile form of *S. commune* such as is shown in plate 522 by Bresadola
(1929). No other characters of diagnostic importance are given in the orig-
inal description. Bresadola has illustrated the species in most of its com-
moner shape variations, but perhaps Rolland's (1910) figure is the most char-
acteristic of the species.

It will be seen from the following list of specimens that in spite of the
scattered stations, *S. commune* is widespread in the United States, and al-
though no specimens are cited, the range probably extends northward well
into Canada. The southern limit of the species in North America, so far as
can be ascertained from the material at hand, appears to be Florida in the
east and California in the west, but it is probable that the species may be
found to grow in Mexico. Further south, the species again appears in Sao
Leopoldo, Rio Grande do Sul, Brazil, although with basidiospores that are
somewhat smaller than are found in the temperate material.

Specimens examined: Maine, Cumberland, 2 collections, on maple and on dead
plum tree, ex herb. Ellis; Massachusetts, Lunenberg, 1883, ex herb. *F. L. Sargent;*
Canton, two collections on apple, December 1931 and May 1932, also on black oak, May
1932, *D. H. Linder;* New York, Buffalo, *G. W. Clinton;* Maryland, Fairhaven, July
1932, *F. K. Sparrow;* Florida, without locality, *Mrs. Curtis;* Ohio, Oxford, September
27, 1907, *Bruce Fink;* Michigan, near Agricultural College (East Lansing?), January
1892, *G. H. Hicks;* Indiana, Scottsburg, on *Nyssa sylvatica,* November 11, 1917,
J. R. Weir, 9365; Kentucky, Conway, December 28, 1920, *Bruce Fink;* Washington,
Montesano, *J. M. Grant;* California, Pasadena, 1911, *L. H. Farlow;* Brazil, Sao Leo-
poldo, 1907, *Rick, 226,* in herb. Theissen.

2. **Schizophyllum radiatum** (Swartz) Fries, Nov. Symb. Myc. in Nova
   Acta Sci., Series 3, 1: 41. 1855.

   Agaricus radiatus Swartz, Prodromus p. 148. 1788.

   Schizophyllum Murrayi Massee, Journ. Bot. **30** (n. ser. **21**): 164. *pl.
   322, figs. 13–14.* 1892.

   Schizophyllum Egelingianum Ell. & Ev., Torrey Bot. Club Bull. **22**: 439.
   1895.

   ?Schizophyllum pavonium Ellis, nom. nud. in Murrill, Mycologia **4**: 214.
   1912.

   Schizophyllum flabellare E. & K., not Fries, exsiccati publication in
   Nash, G. V., Plants of Florida, 2044.

   PLATE 33, fig. 2–5; PLATE 34, fig. 3; PLATE 35, fig. 9–16

Pileus thin, coriaceous, of two types (a) suborbicular, sessile or substipi-
tate and (b) flabelliform, broadly or digitately lobed, sessile, substipitate, or
stipitate by elongation of the margin of the pileus, both types with a villose
pellicle that may or may not be zonate, white, grayish-white, brownish or
" Wood Brown " to " Fawn " of Ridgway (1912); context 108–414μ thick,
otherwise as in *S. commune*; gills radiating from the point of attachment of
the pileus and interspersed with shorter ones, whitish to brownish, and hirsute
on the abhymenial side, ochraceous to brownish (" Wood Brown ") on hy-
menial side, (400)–760–1160μ long, 36–108μ broad at tip, (81)–117–225μ
broad at the base; abhymenial hairs of two types that are correlated with the
types of pilei, (a) stout, simple, hyaline, thick-walled, long exserted hairs
often incrusted with granules, and (b) stout, hyaline, thick-walled, acutely
short-exserted hairs, 3.5–5.5–(7.2)μ diam., either somewhat swollen ter-
minally or else shortly bifurcate near the apex; basidia narrowly clavate to
clavate, (14)–18–21–(31) $\times$ 2.5–4μ with four slender sterigmata; spores
hyaline, ovoid to ellipsoid and obliquely apiculate, 4–6 $\times$ (1.5)–2–2.5μ.

Mostly saprophytic on woody substrata, but may possibly be parasitic on
sugar cane.

Common in the tropics and subtropics.

Apparently this species has two growth phases, one of which closely re-
sembles *S. commune* in that the fruiting bodies are suborbicular and the gills
are furnished with the same type of abhymenial hairs. While the resemblance
between the two species is close, there is also a subtle difference between
them—the abhymenial hairs of *S. commune,* as seen under a hand lens, are
less closely applied to the gills than are those of *S. radiatum;* moreover, the
abhymenial hairs of the former are rather long exserted and abundant at the
junction of the paired gills, whereas in the latter species such hairs are lacking
(compare fig. 1 and 3, pl. 34). The second phase is readily distinguished
by the thin lobate or somewhat digitate fruiting bodies, and by the acutely
short-exserted abhymenial hairs that are either somewhat swollen or else
furcate near the tip (fig. 13, pl. 35). Were it not for the fact that the two
phases grow together in the same colony (fig. 3, pl. 33) and one has been
observed to grow from the place previously occupied by the other, it would
appear that the phases represented two distinct species. Since both phases

reacted quite differently in culture from *S. commune*, and also since the spores of the two phases are identical, but different from *S. commune*, there is little doubt as to their identity.

S. Murrayi as pictured in the plate by Massee is undoubtedly a synonym of this species in spite of the fact that that author stated that it produces ". . . large globose, echinulate vinous spores." Similar spores have been observed by the writer in all of the larger species of this genus, and in most cases they are the conidia of either *Penicillium* or *Aspergillus*. *S. Egelingianum*, based on immature though fruiting material, also falls into synonymy.

The type locality of *Agaricus radiatus* of Swartz is Jamaica, while Fries in transferring the species to *Schizophyllum* cites a collection made at Mirador, Mexico, by Liebmann. Fortunately the spores and microscopical characters of the specimens from the two localities agree in all details; hence no question need arise concerning the identity of the species. The structure of the fruiting bodies, but more especially the shape and size of the spores of the majority of specimens from the tropics, agree with the structure and spores of the specimens that are assumed to be typical. However, the spores of one specimen, labelled *Lactea brunneola* var. *brasiliensis* Bres., from Brazil, are noticeably smaller than the typical spores of the species (compare fig. 10 and 11, pl. 35). Since, however, there is some variation in the size of the spores, even from the same fruiting body, it seems best to consider this specimen as belonging here.

Specimens examined: GEORGIA, Darien, *Ravenel,* in Fungi Americana 786 as *S. flabellare;* FLORIDA, Eustis, Lake County, *G. V. Nash, 1830* and *2014;* LOUISIANA, Covington, December 1919, *G. Arsene, 14253b;* CALIFORNIA, Stanford University, Santa Clara County, *E. B. Copeland* in Baker, C. F., Pacific Coast Fungi 3745; MEXICO, Mirador, *Liebmann,* authentic material; Guanajuato, Montagnes de Santa Rosa, October 1898, comm. *Prof. Alfredo Duges;* near Monterey, on *Magnolia mexicana,* June 1895, *Egeling,* type of *S. Egelingianum;* GUATEMALA, vicinity of Quiriguá, Departmento Izabal, alt. 75–225 m., May 15–31, 1922, *P. C. Standley, 24156,* in Plants of Guatemala; HONDURAS, Ceiba, December 22, 1915, *F. J. Dyer, 18;* PANAMA, Barro Colorado on *Iriartea* palm, January 22, 1932, *R. H. Wetmore;* BAHAMA ISLANDS, Nassau, June 28, 1904, *T. Barbour;* Abaco, Marsh Harbour, July 19, 1904, *G. M. Allen;* CUBA, Cuba Orientalis, 1856–57, *C. Wright;* Limones Garden, on sugar cane, February 4, 1924, *W. H. Weston Jr.* in herb. Weston; JAMAICA, Mandeville, February 1909, *A. E. Wight, 44* and *99;* Kingston, Hope Garden, on dead *Persea americana,* January 29, 1932, *R. H. Wetmore;* SWAN ISLAND, April 1912, *Nelson;* PORTO RICO, Naranjito, November 26, 1915, *Bruce Fink;* Rio Piedras, *J. R. Johnston;* VENEZUELA, Puerto Zamura, 1887, *Jas. Ward, 67* in herb. Patouillard; SURINAM, without data, ex. herb. Schweinitz in Curtis Herb.; FRENCH GUIANA, vicinity of Cayenne, July 14, 1921, *W. E. Broadway* in Plants of French Guiana, 809; ECUADOR, Province of Azuay, Cordillera Oriental, 2000 m., *Rimbach,* in herb. Patouillard; vicinity of Loja, September 29–October 3, 1918, *J. N. Rose* et al, *23763* in U. S. Dept. Agriculture, Gray Herbarium, U. S. National Museum, and New York Botanical Garden, Explorations in South America; BOLIVIA, vicinity of Canamina, 4000 feet elevation, July 19, 1921, *E. O. White, 528;* BRAZIL, Porto Alegre, Rio Grande do Sul, 1912, *W. Herter* in Sydow, Fungi Exotici Exsiccati, 302; no data but labelled *Lactea brunneola* var. *brasiliensis* Bres. in herb. Theissen.

3. **Schizophyllum brevilamellatum** Linder sp. nov.

PLATE 33, fig. 1; PLATE 34, fig. 6; PLATE 35, fig. 7–8

Pilei crassi, coriacei, dense villosi, albo-cinerii vel dilute "Wood Brown," sessiles, imbricati; lamellis longitudinaliter fissis, brevibus, 200–380μ longis, ad bases 180μ crassitudine; contexto denso, hyalino, 520–1300μ crassitudine; pilis abhymenii numerosis, longe exsertis, simplicibus vel furcatis, 3.6–4.5μ diam.; basidiis anguste clavatis, 18–22 × 2–2.5μ; sporis hyalinis, ellipsoideis obliquiterque apiculatis, 6–7.5 × 2.5–3μ.

Fruiting bodies thick, coriaceous, densely villose, grayish-white to dilute "Wood Brown," dimidiate, imbricate; context as in S. commune, 520–1300μ thick; gills under the hand lens appearing as velvety ridges, longitudinally cleft, the resulting pairs distant, 200–380μ long, 180μ thick at the base; abhymenial hairs numerous, long-exserted, simple or furcate at the tips, 3.5–4.5μ diam.; basidia narrowly clavate, 18–22 × 2–2.5μ spores hyaline, ellipsoid and obliquely apiculate, 6–7.5 × 2.3–3μ.

On dead wood. Venezuela.

This species, although superficially resembling the common S. commune, can be separated from that by the short gills which are only one-half to one-third as long as the context is thick. The basidiospores are of the S. radiatum type but of greater size. The other character that appears to separate the species from the preceding ones is the close imbrication of the pilei, but since the members of the genus are so variable in this respect, it seems best not to stress the manner of growth.

4. **Schizophyllum fasciatum** Patouillard, Journ. de Bot. 1: 170. 1887.

Schizophyllum mexicanum Patouillard, Ibid. p. 171.

PLATE 33, fig. 9–10; PLATE 34, fig. 2; PLATE 36, fig. 1–5

Pileus thin, coriaceous, suborbicular, reniform, entire to digitately lobed, sessile to short-stipitate, densely villose, radiately sulcate near the margin, concentrically zonate with alternate light and dark zones, "Pallid Neutral Gray" to light or deep "Benzo Brown"; context dense, 144–468μ thick, hyaline, bordered at the periphery by a narrow subfuscous zone and closely covered by the loosely intertwined hyaline to fuscous hairs of the pellicle; gills longitudinally cleft and radiating from the point of attachment, "Light Drab," "Pallid Neutral Gray" to deep "Benzo Brown," 400–920μ long, 48–54μ thick near the tip, 144–225μ thick at the base; abhymenial hairs dilute fuscous, geniculate and irregularly short-branched near the apices or else geniculate or wavy in the younger hairs, 2.5–3.6μ diam.; cystidia awl-shaped, hyaline or very dilutely colored, few to many and projecting beyond the basidia, 36–54 × 3.5–4.5μ; basidia clavate to broadly clavate, 13–20 × 4μ; spores elongate ellipsoid, 7 × 2μ.

On dead wood. Tropical.

This species is readily separated from all others by the presence of cystidia and by the colored, geniculate and branched abhymenial hairs (fig. 4, pl. 36). The pilei vary in size from 2× 2.5 cm. to 4 × 6 cm.

S. mexicanum as it is represented in the Patouillard herbarium differs from S. fasciatum only in the lighter color of the fruiting bodies and by the fact that cystidia are less numerous, both characters that are very variable.

Specimens examined: TEXAS, near Victoria, November 1830, *Berlandier*, in Herbarium Berlandierianum Texano-Mexicanum; MEXICO, Atures, August 9, 1887, *A. Gaillard* in herb. Patouillard; Vera Cruz, August 1864, *Sallé;* Iuxtla, 1856, *Sallé*, as *S. mexicanum;* Cordova, December 1854, *Sallé, 149*, as *S. umbrinum;* San Louis Potosi, *Palmer;* CUBA, Central Trinidad, Izuaga, on *Dichrostachys nutans*, Nov. 25, 1924, *Weir & Feris;* VIRGIN ISLANDS, Løvenland, December 1905, *C. Raunkiaer;* St. John, February 14, 1906, *C. Raunkiaer;* TRINIDAD, B. W. I., April 1888; *L. Savage*, in herb. Patouillard as *S. mexicanum;* NICARAGUA, 1853–56, *C. Wright* in U. S. North Pacific Exploring Expedition, as *S. commune.*

5. **Schizophyllum umbrinum** Berkeley, Hooker's Journ. Bot. 3 : 15. *pl. 1, fig. 1.* 1851.

   Schizophyllum multifidum var. *digitatum* Ellis & Macbride, Univ. Iowa Bull. 3 : 194. 1896.

   PLATE 33, fig. 8; PLATE 34, fig. 4; PLATE 36, fig. 6–11

Fruiting bodies small, less than 1 × 1.5 cm., rather thick, suborbicular to flabelliform, lobate to deeply cleft, subsessile to laterally stipitate, the stipe when present sometimes strigose with white hairs, solitary (?) or gregarious, dark brown; context hyaline or somewhat brownish tinged, bordered on the upper surface by a narrow fuscous zone and covered by a pellicle of loosely intertwined fuscous hyphae, the hyphae of the context with walls that are thicker than the diameter of the lumen, 350–525μ thick; gills dark brown, longitudinally cleft, short and tightly inrolled, the context brownish, 350–550μ long, 60–75μ thick near the tip, 145–180μ thick at the base; abhymenial hairs fuscous, closely applied, undulate or spirally undulate, rarely somewhat contorted, 3.5–5.5μ diam.; basidia broadly clavate and tapering slightly towards the base, the young ones with very thick hyaline or dilute fuscous walls and frequently surmounted by a brown secretion, 15.5–23.5 × 4–5μ, the older basidia thin-walled and projecting beyond the hymenium, 19–26 × 4–5μ; spores hyaline, ellipsoid and obliquely apiculate, 4.5–6 × 2–2.5μ.

On dead wood. Tropical.

Because of its small size and its brown color, this species is readily distinguished from those that have preceded. The narrow lumens of the hyphae of the context give that tissue the appearance of a gelatinous matrix that has been run through by slender threads, with here and there a scattering of knots where some of the hyphae have enlarged (fig. 9, pl. 36). The abhymenial hairs of the type collection in the Curtis Herbarium, as shown in figure 10 of plate 36, are conspicuously undulate, whereas those of the Nicaraguan material (type collection of *S. multifidum* var. *digitatum*) and of the Swan Island specimens are not only somewhat more slender and of lighter color, but also less conspicuously undulate; also the abhymenial hairs of the Nicaraguan material are crowded by the deposits of almost square crystalloid bodies. In addition, the latter material differs by the finger-like elongations of the lobes of the pileus. Such differences, however, seem to represent variations induced by differences in environmental factors.

Specimens examined: CUBA, no locality other than Cuba Orientalis, 1856–57, *C. Wright,* in Plantae Cubensis Wrightianae; SWAN ISLAND, April 1912, *Nelson;* NICARAGUA, on logs in low land, *C. Wright,* U. S. North Pacific Exploring Expedition;

Ometepe, January–February 1893, *C. L. Smith,* in Central American Fungi, 54, type collection of *S. multifidum* var. *digitatum.*

6. Schizophyllum Leprieurii Linder sp. nov.

PLATE 33, fig. 7; PLATE 34, fig. 5; PLATE 36, fig. 12

Pilei 1 × 1 cm. vel minores, atro-fusci, subcrassi, coriacei, dense et minute villosi; contexto denso, dilute fusco et ad confine superiore subfusco vel fusco, 360–440μ crassitudine; hyphis pelliculae subfuscis vel fuscis, laxe implexis; lamellis atrofuscis, longitudinaliter fissis, brevibus, arte incurvatis, 280–440μ longis, ad bases 162–216μ crassitudine, ad apices 54–72μ crassitudine; pilis abhymenii subfuscis praeter apices ubi hyalinis ramosisque, ramis undosis, contortulis vel nonnihil glomeratis, 1.8–2.7μ diametro; basidiis juvenibus parietibus crassis, clavatis 27–36 × 4.5μ; sporis non observatis.

Fruiting bodies small, 1 × 1 cm. or less, dark brown, rather thick, coriaceous, suborbicular to flabelliform, lobate or digitate, substipitate or stipitate; context light brown, bordered on the upper surface by a narrow fuscous zone, and covered by a pellicle of loosely interwoven fuscous hyphae, 360–440μ thick; the hyphae of the context the same as those of *S. umbrinum;* gills dark brown, longitudinally cleft, short and tightly coiled, 280–440μ long, 162–216μ thick at the base, 54–72μ thick near the apex; abhymenial hairs light fuscous, appressed except near the tip of the gill where they are branched, the branches hyaline, undulate, somewhat coiled or contorted, 1.8–2.7μ in diameter; young basidia clavate with thick lateral and terminal walls, 26–36 × 4–5μ; spores not observed.

Presumably occurring on dead wood. French Guiana.

In color and size, and in the structure of the context and basidia, this species closely resembles *S. umbrinum,* but may be separated from that species on the basis of the more slender abhymenial hairs which are branched near the tips.

Specimen examined: FRENCH GUIANA, *Leprieur, 1003,* ex. herb. Richard, in Patouillard Herbarium. TYPE.

SUMMARY

Six species of *Schizophyllum* from the Western Hemisphere are described, illustrated, and their synonyms listed. Of the six species, two, *S. brevilamellatum* and *S. Leprieurii,* from Venezuela and French Guiana, respectively, are described as new.

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EXPLANATION OF PLATES

PLATE 33

All photographs, with the exception of fig. 3, were made from dry herbarium material. The scale accompanying each figure represents 1 cm.

FIG. 1. *Schizophyllum brevilamellatum*. Note the rather thick fruiting bodies and their closely imbricate arrangement. Type material from Venezuela.

FIG. 2. *Schizophyllum radiatum* from Guanajuato, Mexico, showing a large, deeply cleft fruiting body with a distinct, if short stipe. Upper and lower sides of the same specimen.

FIG. 3. *Schizophyllum radiatum* from Panama illustrating the *S. commune* type of fruiting body (second from the top) and the almost resupinate typical fruiting bodies.

FIG. 4. *Schizophyllum radiatum* collected at Mirador, Mexico, by Liebmann and authentic as typifying the Friesian concept of the species.

FIG. 5. *Schizophyllum radiatum* from Brazil.

FIG. 6. *Schizophyllum commune* from Sao Leopoldo, Brazil, Rick no. 226.

FIG. 7. *Schizophyllum Lepricurii*. Type material from French Guiana and showing the finger-like elongations of the pileus.

FIG. 8. *Schizophyllum umbrinum* from Swan Island. Note the pilose stipe and the lobing of the pileus.

FIG. 9. *Schizophyllum fasciatum* authentic material from Mexico in the Patouillard Herbarium.

FIG. 10. *Schizophyllum fasciatum* from the Virgin Islands, illustrating the transition from the suborbicular type of fruiting body to the ramose, digitate type.

PLATE 34

All figures are semidiagrammatic and drawn to the same scale with the aid of a camera lucida.

FIG. 1. *Schizophyllum commune*. A section of a pileus to show the length of the gills in relation to the width of the context, the long exserted hairs, and the numerous hairs at the junction of the split gills. The splitting of the left hand gill by the breaking down of the hyphae in the center of the context is indicated by the dark irregular lines. Material from Scottsburg, Indiana.

FIG. 2. *Schizophyllum fasciatum*. A section of the pileus of St. Croix material. At the extreme left of the figure is shown a very young gill. From the loose arrangement of the hyphae and the distance between the halves of the gill, it will be seen that there is little evidence that the gills are formed as a result of folding.

FIG. 3. *Schizophyllum radiatum*. A section of a typical fruiting body characterized by the acutely short-exserted abhymenial hairs. The gill on the left has been completely separated, as has the context, by the breaking down of the hyphae.

FIG. 4. *Schizophyllum umbrinum*. A section of the pileus illustrating the relation of the length of the gills to the context, the closely inrolled halves of the gills, and a young gill with its uncrowded hyphal elements. In the right half of the section are

parts of two gills separated by the shorter halves of a single young one, giving to the two sets an appearance of being obliquely arranged.

Fig. 5. *Schizophyllum Lepricurii.* A section of a pileus, the brownish context of which is indicated by the more densely stippled area. Although not shown in the figure, the gills of this species also have the appearance of being obliquely arranged.

Fig. 6. *Schizophyllum brevilamellatum.* A section of the pileus to show the very thick context and the short, distant halves of the gills.

PLATE 35

All drawings are made with the aid of a camera lucida from material mounted in lactophenol. The figures (2, 7, 13, 16) of sections of the gills are represented, after reduction, at a magnification of approximately × 500 as is shown by the lower scale beneath the signature; all others are shown magnified approximately × 1125, indicated by the upper scale beneath the signature.

Schizophyllum commune, figures 1–6

Fig. 1. Spores from material collected in Canton, Massachusetts.

Fig. 2. Section of the tip of a gill to show the hymenium and the stout, granular incrusted, long exserted abhymenial hairs.

Fig. 3. A young and a discharged basidium from European material in Flora Hungarici Exsiccati Cent. V., fungi 50, no. 410.

Fig. 4. Spores from material collected by Rick (no. 226) at Sao Leopoldo, Brazil.

Fig. 5. Spores from material collected at Pasadena, California, by L. H. Farlow.

Fig. 6. Spores from material described under figure 3.

Schizophyllum brevilamellatum, figures 7–8

Fig. 7. A section of approximately one-half of a gill to illustrate the relative thickness of the gill, and the simple and furcate hairs of the abhymenium.

Fig. 8. Spores and a young basidium. Type material from Venezuela.

Schizophyllum radiatum, figures 9–16

Fig. 9. Spores from type material of *S. Egelingianum.*

Fig. 10. Spores from material collected in Eustis, Florida, in Nash, G.V.—Plants of Florida, no. 2044.

Fig. 11. Spores from a collection made in Brazil and labelled *Lactea brunneola* var. *brasiliensis* in Theissen Herb.

Fig. 12. Spores from Panama material.

Fig. 13. Tip of a gill of typical material to show the differentiation of the subhymenial layer and the acutely short-exserted, swollen or furcate abhymenial hairs. From collection illustrated in fig. 3, pl. 33.

Fig. 14. Spores from material collected at Huigra, Ecuador.

Fig. 15. Spores and cystidium-like basidium from Panama material.

Fig. 16. The tip of a gill from the S. commune type of fruiting body illustrated in fig. 3, pl. 33. The abhymenial hairs resemble those of *S. commune*, but the spores of this fruiting body (fig. 15 above) are identical with the spores from typical material.

PLATE 36

The drawings are all made from material mounted in lactophenol. Figures 1, 6, 10, and 12 are shown at a magnification of approximately × 500; all other figures are magnified approximately × 1125.

Schizophyllum fasciatum, figures 1–5

Fig. 1. Section of the tip of a gill to show the branched light fuscous abhymenial hairs and the cystidia. Material from St. Croix.

Fig. 2, 3. Basidia and cystidia from St. Croix material.

Fig. 4. An abhymenial hair which illustrates the characteristic geniculation and branching.

Fig. 5. A portion of the context to show the compactness of the tissue. The lumen of the hyphae is represented by the densely stippled areas.

Schizophyllum umbrinum, figures 6–11

Fig. 6. A part of the context of the basal portion of a gill with the accompanying young basidia. Note the very narrow lumen, and the direct connection between the hyphae of the gill context and the basidia. Material from Cuba.

Fig. 7, 8. Young and mature basidia. The young ones are readily distinguished by their thick, almost hyaline walls.

Fig. 9. A part of the context of the pileus. The lumen is about one-third the diameter of the hypha, but isolated enlarged hyphae occur scattered throughout the context.

Fig. 10. The tip of a gill showing the fuscous, undulate abhymenial hairs closely appressed to the back of the gill.

Fig. 11. Spores from Cuban material in C. Wright, Plantae Cubensis Wrightianae.

Schizophylum Leprieurii, figure 12

Fig. 12. The tip of a gill to show the branched apices of the terminal abhymenial hairs. These hairs are more slender than are those of *S. umbrinum.* Type material from French Guiana.

LINDER SCHIZOPHYLLUM
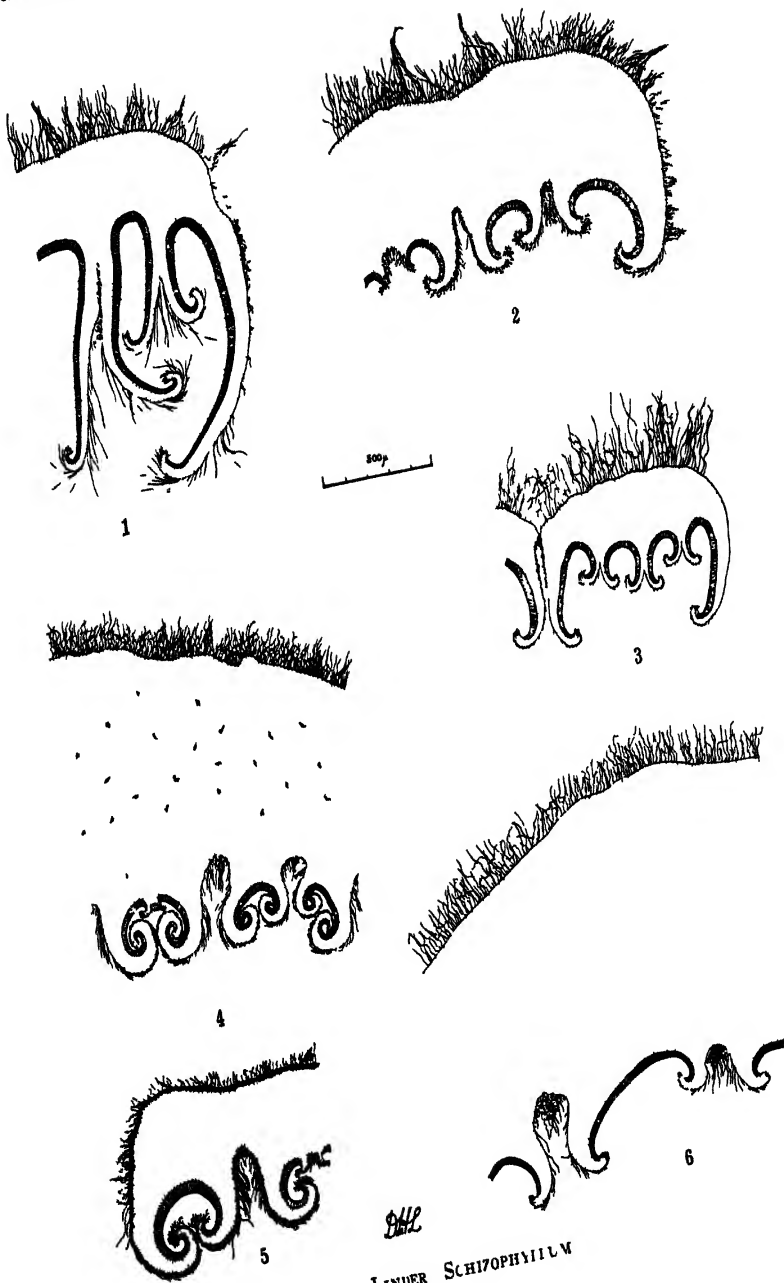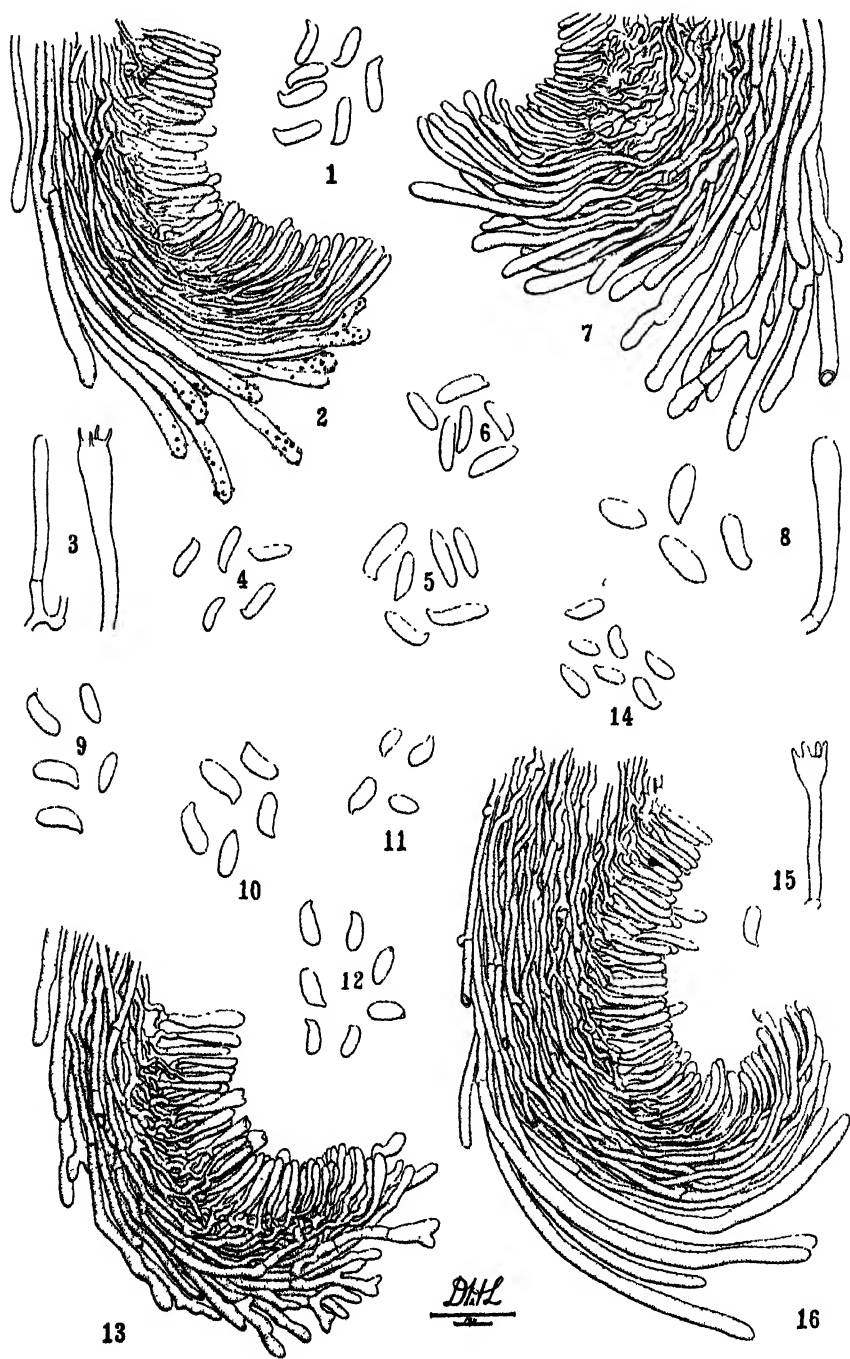
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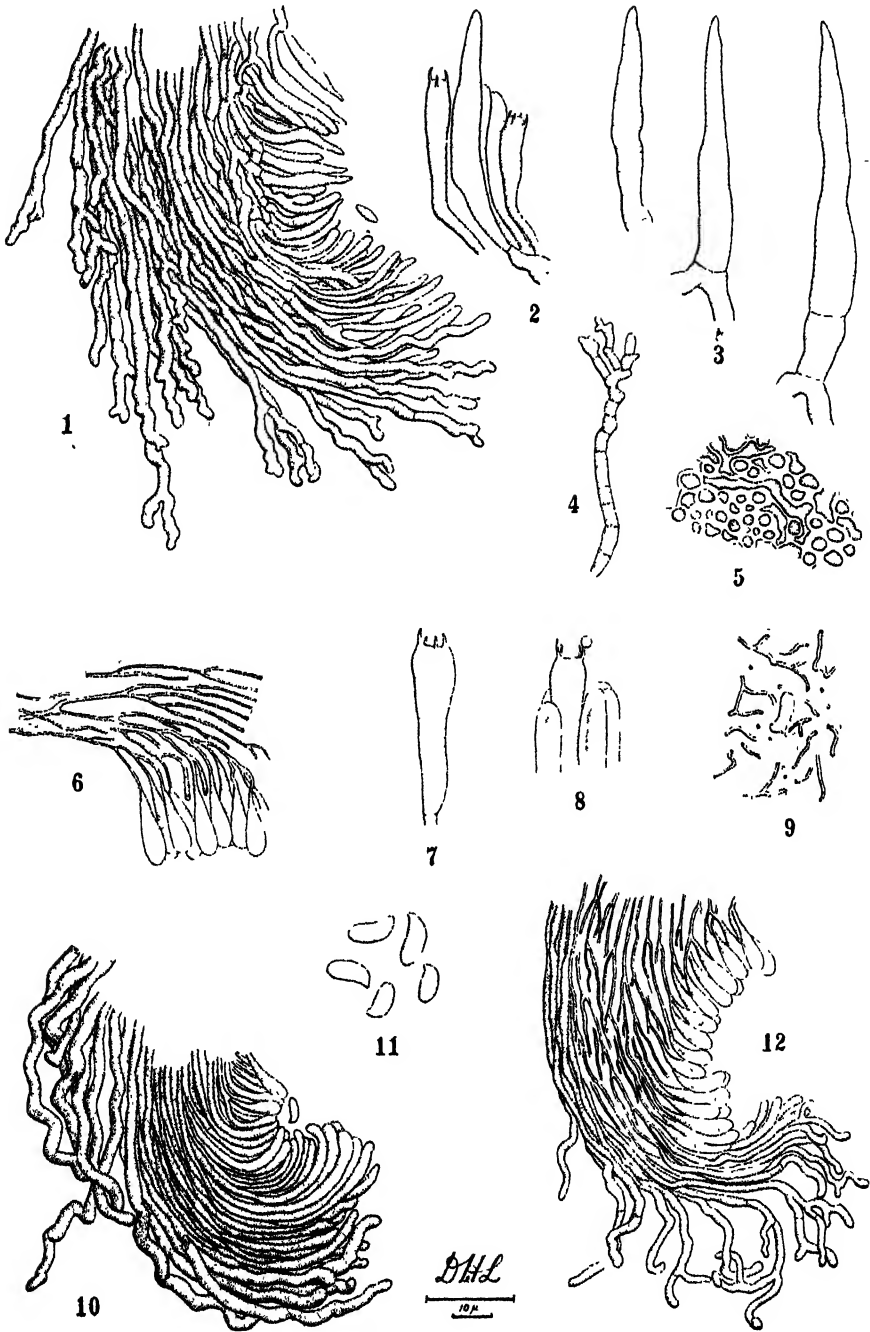
LINDER: SCHIZOPHYLLUM

STRUCTURE AND DEVELOPMENT OF THE TOBACCO LEAF

George S. Avery, Jr.

The four periods in plant development which might be included in an ideal study of the developmental anatomy of a seed plant have been discussed in an earlier paper, and the second period, involving the seedling, has been considered in some detail (Avery, 1933). The third period, one of marked vegetative increase, includes leaf, stem, and root ontogeny. It is characterized by continued embryology at all growing points, and the resulting successive origination of new organs. Because of its greater importance to our knowledge of the tobacco plant, as well as its bearing on our rather sketchy knowledge of leaf development, only leaf ontogeny has been included here.

It is a curious fact that in the numerous papers dealing with leaf structure, none attempts to trace in any detail the changes which take place in the shape of a leaf, as well as in its tissues, from the time it first appears as a lateral projection from the growing point of the stem, until it has become mature. This study is confined principally to developmental morphology, correlating tissue ontogeny with external development in the hope that the final result may be a somewhat clearer picture of how a tobacco leaf acquires its characteristic shape and form. An attempt has been made to determine the origin of tissues, the plane and direction of cell divisions and cell enlargement, the duration of such divisions and enlargement, and the resulting tissue strains and their influence on cell shape, character of tissues, etc. Huxley's (1932) technique has been applied to the leaf for studies in relative growth.

MATERIALS AND METHODS

Three varieties of *Nicotiana tabacum* L. were used for studies on leaf ontogeny—i.e., Havana Seed, Cash, and Cuban Shade. The tips of half-grown plants were removed at various times of day and night, some being placed in formal-acetic-alcohol and others in Bouin's solution. Still others were dissected out under a binocular microscope while still fresh. The presence of copious hairs renders the individual embryonic leaves of limited use in the fresh condition. Stem tips of plants of the Havana Seed variety, placed in Bouin's solution at 12:45 a.m., showed the greatest number of nuclear and cell divisions; all detailed drawings were made from these with the aid of a microprojector.

Some of the freshly dissected out embryonic leaves were later killed, dehydrated in a series of alcohols, stained with crystal violet in clove oil, cleared

in xylol, and mounted in balsam in toto. Others were imbedded in paraffin and cut parallel to the surface of the embryonic lamina, at a thickness of 8 microns. Such material was of assistance in determining shape of leaf primordia, relative stages of development of principal veins, etc. Entire stem tips with 12 to 16 accompanying leaves (killed and dehydrated as mentioned above) were imbedded by the paraffin method, cut in serial transverse sections at 12 microns, and stained with safranin and fast green. With their successively older primordia, they provided the different ages of material for study.
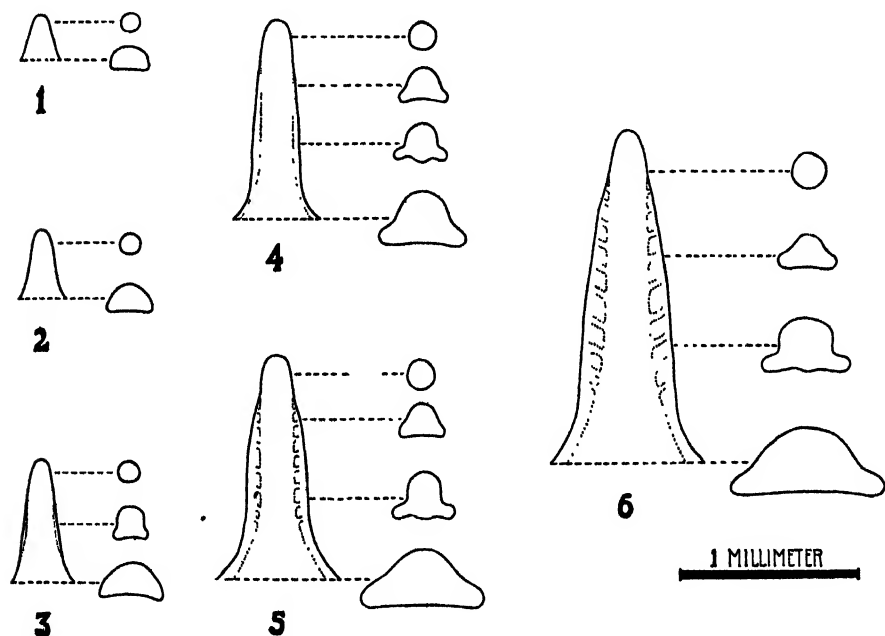
Fig. 1–6. Diagrams of longitudinal and transverse sections of foliage leaf primordia, in six successive stages of development. Fig. 1, the primordium in early development (see fig. 7, a, b). Fig. 2, the primordium next older than that shown in fig. 1 (see fig. 8, a, b). Fig. 3, next below the primordium shown in fig. 2. The small lateral ridges appearing about midway on the primordium are forerunners of the lamina (see fig. 9, a, b, c; also fig. 13). Fig. 4, next older than that shown in fig. 3. Development of the lamina is definitely started (see fig. 10, a, b, c). Fig. 5–6, successively older primordia, showing further expansion of the lamina and the beginning of development of the main lateral veins (see fig. 11, a, b, c, and fig. 12, a, b, c). Dotted lines in fig. 3–6 represent external boundaries of midrib and lateral veins, and not the provascular portion.

LEAF ONTOGENY

External developmental morphology

Leaves are being successively originated at the growing point throughout the active vegetative period, and are laid down spirally with a 2/9 phyllotaxy.

When 0.2 mm. long the young leaf increases rapidly in length from a pyramidal but somewhat flattened mass of embryonic tissue (fig. 1) to a dis-
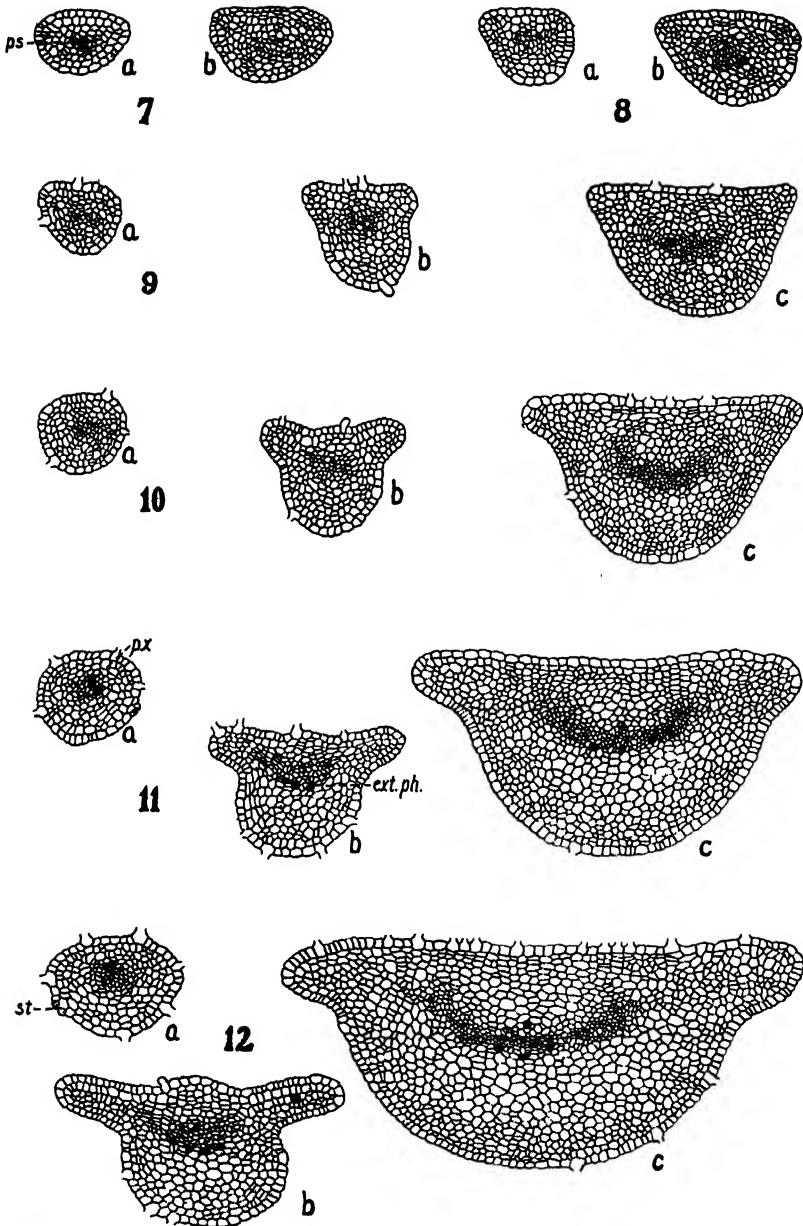
Fig. 7–12. Fig. 7–8, transverse sections of middle (*a*) and basal (*b*) portions of the primordia shown in fig. 1 and 2. The provascular strand (*ps*) shows in the middle portion of the younger primordium (*7a*), indicating greater relative maturity of tip. Fig. 9–12. Drawings "*a*" represent transverse sections 125 to 150 μ below the tip; "*b*," about midway between base and tip, and "*c*," the basal portion. They represent the successive primordia shown in fig. 3–6. Note the differentiated protoxylem (*px*) and external phloem (*ext. ph.*) in fig. 11, *a, b, c*. This primordium is 1.2 mm. in length. In fig. 12 *a*, the nearly mature tip shows a stoma (*st*).

tinctly linear primordium at a length of 1.1 mm. (fig. 2, 3, 4). Though largely midrib primordium up until this time, the lamina has become discernible in the form of lateral bulges on opposite sides of the midrib (fig. 4). The development of lateral veins follows (fig. 5, 6). Connecting veins first differentiate at the tip (fig. 18), then successively toward the less mature base of the leaf (fig. 19). The tip matures rather early, but growth continues basipetally as long as the leaf develops (fig. 20, 21, 28–31). There is, therefore, a distinct age-gradient from the mature distal end of the leaf to its actively growing base, where it is attached to the axis. Enlargement of the lamina takes place at a greater rate in the basal and central portions of the leaf, particularly the latter, resulting in the ovate shape of the leaf (fig. 31a).

Epidermal hairs begin to appear when the primordium is approximately 0.5 mm. long, accounting for the densely hairy condition in earlier stages of development. This condition becomes less pronounced as the distance increases between hairs, due to expansion throughout the lamina.

Origin of the primordium, midrib, and large lateral veins

The primordial leaf is at first a mere hump of embryonic tissue projecting laterally from the growing point of the stem. It derives its epidermis from the protoderm and its parenchyma from the ground meristem; soon it itself becomes a distinct center of growth. A period of general and rapid meristematic activity characterizes its development up to the time it is 2–3 mm. in length. It is little more than embryonic midrib (fig. 1, 2, 3, 7, 8, 9b), however, until it is 0.6 mm. or more in length, at which time lamina development is initiated (fig. 4, 10b).

The addition of cells at its apex may be traced to the activity of a single sub-epidermal cell, which appears in longitudinal median section, as shown in fig. 17, "A." This cell gives rise to the provascular bundle of the midrib, the bundle sheath, etc. When the primordium is 2–3 mm. in length (fig. 6, 18, 19), apical growth ceases and elongation of the midrib continues basipetally, together with basipetal enlargement of the lamina, which is described later.

The principal lateral veins first differentiate (as provascular strands) when the primordium is about 1.25 mm. long (fig. 5). They extend from the midrib to near the margin (fig. 18, etc.), having their origin in the " middle mesophyll " (fig. 16) as the latter develops at the edge of the lamina. They increase in length by intercalary growth, and thus keep pace with the enlargement of the lamina. The differentiation of the finer vascular network is described in the section upon cellular differentiation.

Origin and development of the lamina

When the primordium is approximately 0.6 mm. long, two ridge-like projections appear, one on either side of its adaxial half (fig. 3, 4). These extend a short distance toward base and tip, and constitute the beginning of the lamina. These ridge-like projections are initiated by the activity of

a row of subepidermal cells ("marginal meristem"), which extends along
either side of the midrib primordium. The behavior of this subepidermal
row, which, of course, appears as a single cell in transverse section, is as
follows: Figure 13 shows the subepidermal cell in position A dividing to
form cells in positions A^1 and A^2 (fig. 14). Figure 15 shows A^2 divided, a
new cell wall having been laid down parallel to the epidermis. A^1 above it
(no label) is enlarging and becoming a palisade mother cell. The outer, or
subepidermal daughter cell after the division of A^2, again occupies the orig-
inal position A (fig. 15). The inner daughter cell in position B divides (fig.
16) to form cells in positions B^1 and B^2. The three layers of "middle meso-
phyll" are derived from subsequent divisions of cells occupying the positions
B^1 and B^2. The next division of the cell in position A results in two new
cells occupying the positions A^1 and A^2 (fig. 14). This time A^2 enlarges,
becoming a mother cell of the lower mesophyll, and A^1 divides to form new
cells in the positions A and B. This cycle apparently continues as long as
there is marginal growth. All cells laid down by the marginal meristem are
mesophyll mother cells, and those of the middle mesophyll are potentially
provascular, as indicated above in the description of the origin of veins. All
mesophyll mother cells undergo subsequent divisions throughout the lamina
for some time, and enlargement follows. The epidermis is self-perpetuating.
Its behavior in relation to the mesophyll might be likened to that of periclinal
chimera.

As the primordium approaches 3 mm. in length, marginal growth toward
the distal end stops, along with the cessation of apical growth. At this time
the primordium is 1/30 or less the size of the mature leaf. The tip of the
young leaf becomes mature, as is evidenced by the well-differentiated xylem,
abundant intercellular spaces, the presence of stomata, and a conspicuous
cuticle (fig. 23 a). Marginal growth continues centrally and toward the
basal end, however, together with growth throughout the rest of the lamina,
until the leaf is several centimeters long. The distal portion of the leaf is
therefore relatively more mature, and the gradient from tip to base is a
gradient from senescent to young tissues.

Duration of cell division

The number of cell layers in the lamina, developed as a result of the
marginal activity described above, is usually established when the primordium
is less than 5 mm. long (fig. 23 b), though many leaves do not show it until
later (fig. 25), and some show it on one side of the midrib earlier than on
the other (fig. 24 b). Once established, the number of cell layers remains
the same throughout the life of the leaf (fig. 26, 27, 33, 36). Cell division
ceases first in the epidermal layers, as indicated by their greater size in the
mature leaf, next in the region which becomes spongy mesophyll, and last
in the palisade. A few cells of the spongy mesophyll continue division after
all others have stopped, and give rise to new provascular strands during inter-
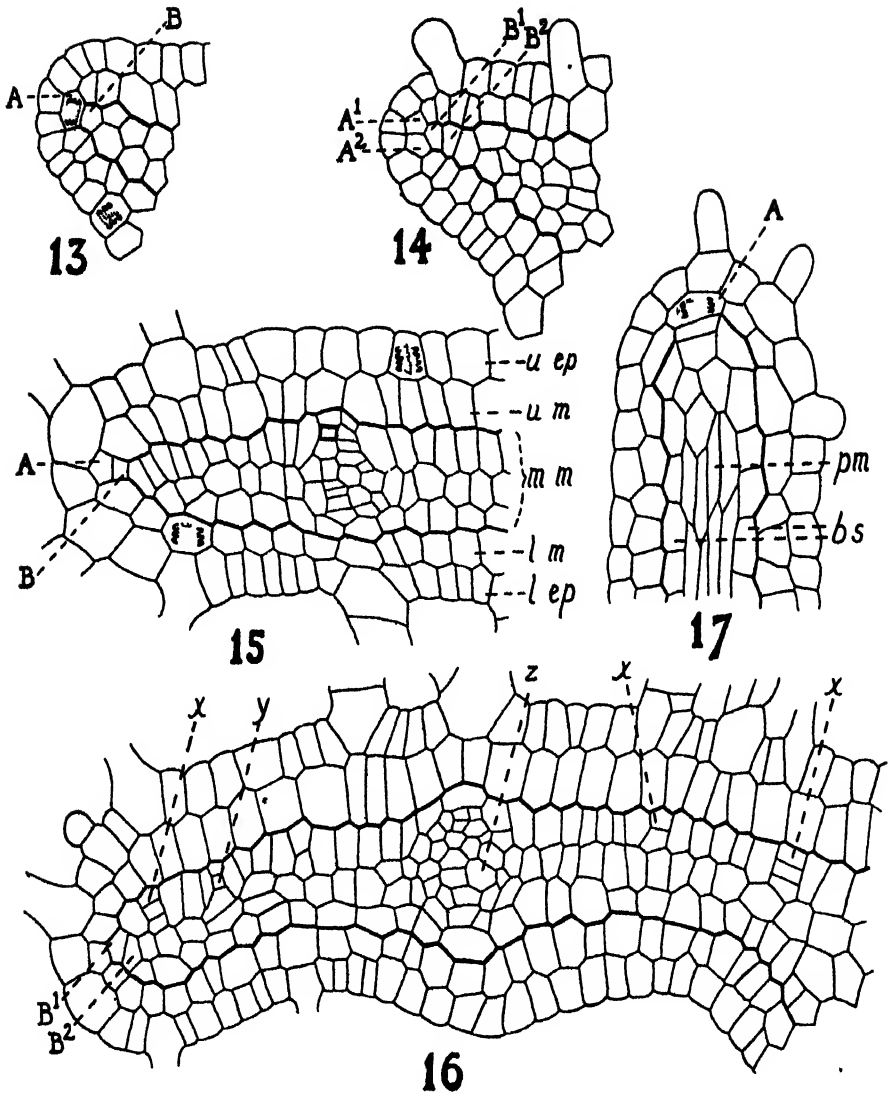
Fig. 13-17. Fig 13-16, transverse sections of the edges of young laminae in various stages of development, showing subepidermal cell divisions Such divisions and subsequent enlargement of the new cells bring about marginal growth of the young lamina. Fig. 13, same primordium shown in fig. 3. Fig. 14, same, see fig. 4. Fig. 15, outer edge of the lamina of the fourteenth leaf below the growing point (see fig. 27) : *u ep*, upper epidermis; *u m*, upper or adaxial layer of mesophyll; *m m*, middle mesophyll; *l m*, lower or abaxial layer of mesophyll; *l ep*, lower epidermis. Fig. 16, same, tenth leaf below the growing point. Note younger stages of the development of veins (*x*) and older stages at *y* and *z*. Fig. 17, longitudinal median section of young primordium, showing how new cells are formed at the apex, in early development, as a result of subepidermal activity. They give rise to the provascular strand of the midrib (*p m*), the bundle sheath (*b s*), etc.

calary growth of the lamina. The external factors which influence a continuation of cell division are numerous, but in the material examined, epidermal cells were no longer dividing, even in the basal and central regions of leaves from 6 to 7 centimeters long (1/5 to 1/6 final size). The degree of maturity of all cells, however, is dependent upon their distance from the tip and their distance from the margin.

The relation of strains and stresses to the origin of the spongy tissue, and the reciprocal influence of the development of this tissue on epidermal cell shape

That there is a slight tension between the mesophyll and the epidermis when the leaf is quite young (a few centimeters long) may be demonstrated by carefully slitting the leaf at the margin. If quickly placed in water, it curves outward. This suggests that the impetus for development comes from the mesophyll, and that the epidermis expands none too readily to meet its demands.

However, cells of the epidermis continue enlarging after those of the *middle* and *lower* mesophyll have ceased to enlarge. As a consequence (having ceased division only a very short time after the epidermal cells), they are pulled apart as the epidermal cells continue to enlarge. This results in the spongy tissue. Cells of the latter exert a reciprocal " pull " on the lower epidermal cells. This pull, being nearly equal in all directions, distorts the lateral epidermal cell walls and makes them appear wavy.

If the leaf continues to increase in size, the palisade cells (which are the last to cease dividing) are pulled apart in a similar fashion by the enlarging upper epidermal cells, though not as markedly as in the case of the spongy tissues. The reciprocal stresses of the palisade on the upper epidermis may effect a similar waviness, though less pronounced, in the lateral walls of upper epidermal cells.

Cellular differentiation in the lamina

Epidermal cells acquire their characteristic depth almost at the beginning, but the wavy outline of their lateral walls does not develop until long after cell division has ceased, as described above. Stomata, although appearing at the mature tips of young leaves less than 2 mm. long, where the epidermal cells have ceased dividing (fig. 11 *a*, 12 *a*), do not appear elsewhere in the lamina until later (fig. 33), for cell division continues longer in all but the distal portion of the lamina. The epidermal hairs are quite diverse. They may be either branched or single-stalked, simple, or capitate and glandular, secreting a viscid, gummy substance.

Cells of the palisade layer begin to appear markedly larger in size almost as soon as the lamina is initiated (fig. 12 *b*, 22 *b*, etc.), but they do not begin to acquire their characteristic depth until the young leaf is 4–5 mm. long (fig. 19, 29, 23 *b*, 24 *b*). From this time on they are markedly columnar in their

appearance (fig. 26, 27, etc.). Air spaces develop between those situated under stomata soon after the latter develop (fig. 36).

The origin of the middle layers of mesophyll has been described. The cells retain their compactness until the leaf is some 8–10 centimeters in length (¼ to ⅓ final size). Most cell divisions have ceased prior to this, and from this time on there is considerable cell enlargement and development of intercellular spaces (fig. 36, 37).
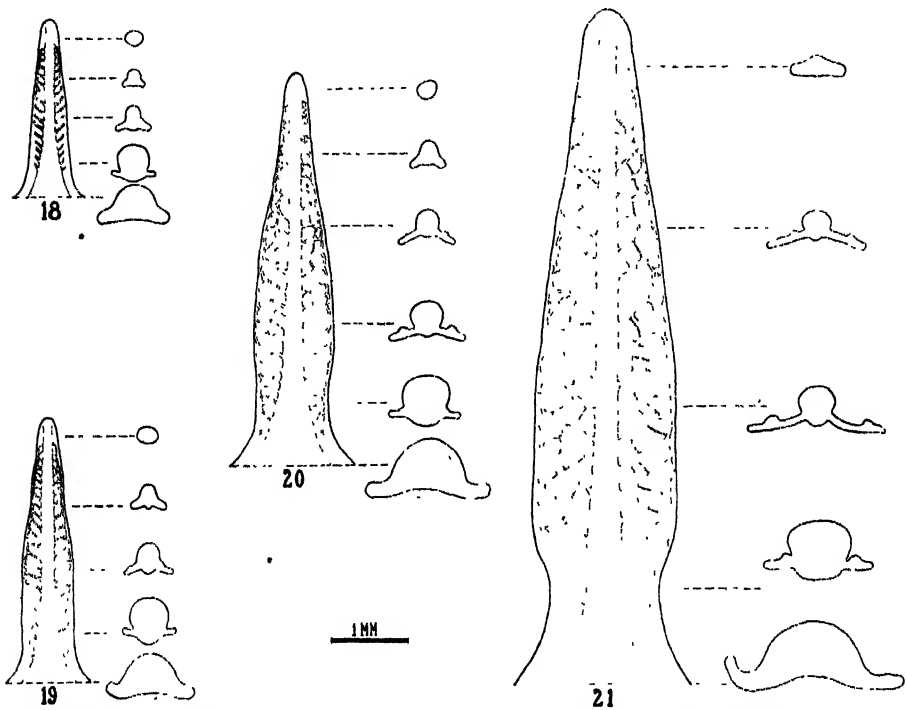
Fig. 18–21. Diagrams of longitudinal and transverse sections of foliage leaf primordia. Four successive stages, following those shown in fig. 1–6. The relatively greater maturity of the tips is indicated by the development of the vascular network at the distal end. Fig. 18, primordium approximately 2.25 mm. long, with completely blocked out provascular system at distal end (see fig. 22 a, b, c). Fig. 19, primordium with network of provascular strands developing downward from distal end (see fig. 23 a, b, c). Fig. 20, primordium 5 mm. in length. Note the further development, toward the base, of the network of provascular strands (see fig. 24 a, b, c). Fig. 21, slightly older primordium with the development of the lamina and system of venation well started (see fig. 25).

The smaller veins arise from the middle mesophyll in diverse ways. A common mode of origin in the young lamina is for a row of cells (a single cell in transverse section), usually in the upper layer of middle mesophyll (fig. 16, x), to divide, with one of the resulting rows undergoing a second division (fig. 16, y). The upper of these three cells, as seen in transverse section, becomes bundle sheath, as do the cells lateral to the lower two. Subsequent divisions of the two lower cells usually give rise to the remainder
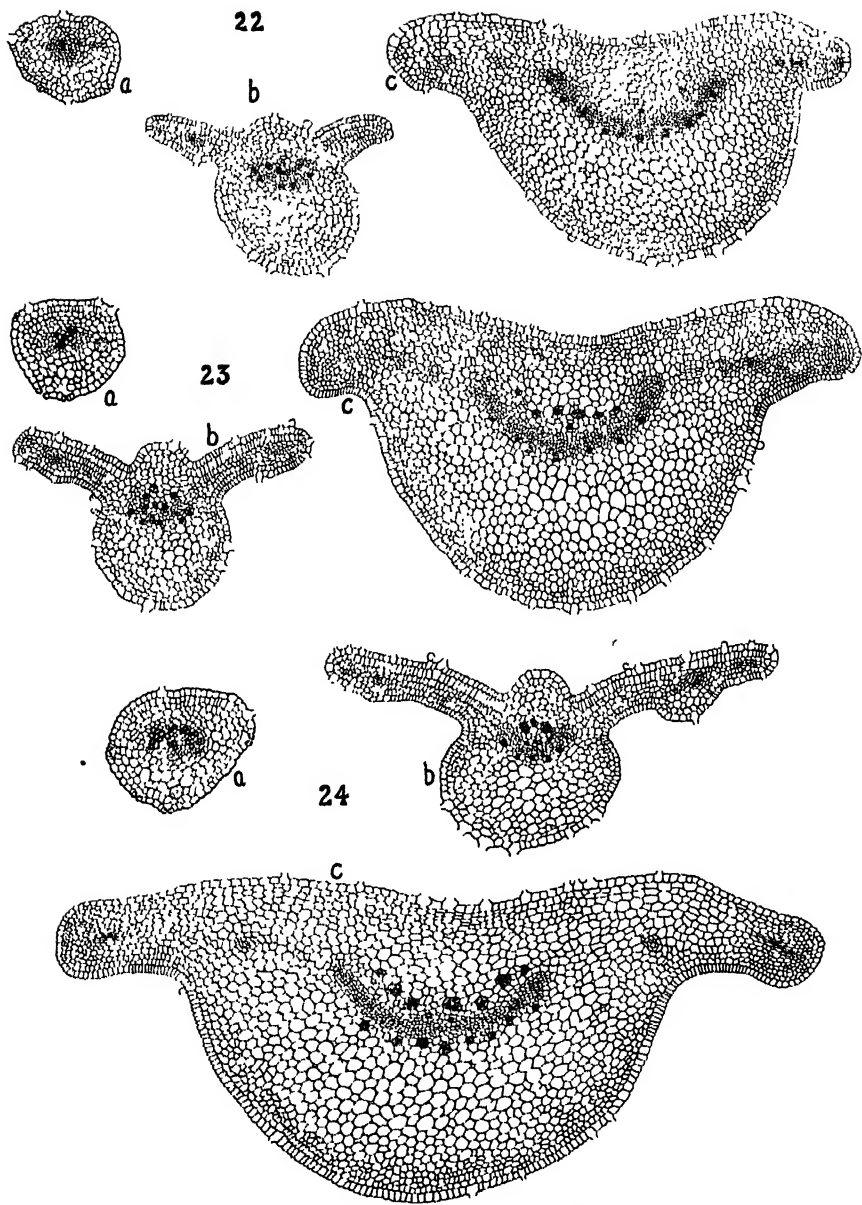
Fig. 22-24. Transverse sections of tip (a, 150 to 200 μ below tip), middle (b), and base (c) of the successively older primordia shown in fig. 18-20. Fig. 22, numerous stomata and a slightly thickened cuticle appear near the distal end of the primordium, indicating greater maturity of tissues than in other portions of the leaf. Palisade cells show marked enlargement (b). Internal phloem is first discernible (b, c) at this stage of development (primordium 2.25 mm. long). Fig. 23 shows still greater differentiation at the tip. The intercellular spaces are becoming increasingly evident, and a cell has appeared bearing calcium oxalate crystals. The internal phloem shows greater differentiation near the base (c). Palisade cells in the middle portion of the leaf are assuming their characteristic shape (primordium 3.33 mm. long). Fig. 24, with completely mature tip (a), an obviously less mature central portion (b), and an actively growing basal portion (c) with but a small amount of differentiated xylem. Note the three layers of middle mesophyll in the lamina on one side of the midrib and but two on the other.

of the bundle, unless it is to be a larger one, in which case adjacent cells of the middle mesophyll on the lower side of the bundle may become involved (fig. 16, *s*). Provascular strands arise in the central and basal portions of the lamina as long as that particular part of the lamina undergoes marked expansion, thus keeping a portion of the spongy mesophyll meristematic over a longer period of time than any other tissue of the leaf. Except for these special portions of the spongy tissue, it closely follows the epidermis in cessation of cell divisions, as pointed out above.

The xylem and phloem differentiate in the usual manner in all veins of the leaf, the earliest conspicuous differentiation being that of the xylem and external phloem at the tip of the midrib, when the primordium is little over 1 mm. long (fig. 5, 11 *a*, *b*, *c*). The internal phloem is not apparent, however, until the primordium is approximately twice this length. It differentiates rapidly after this time (fig. 22 *b*, *c*), but is not distributed throughout the veins of the leaf as is the external phloem. It is confined to the midrib and the main lateral branches (fig. 41). The smaller veins making up the ultimate vascular network never possess it (fig. 40). In leaves borne about midway on the stalk, and exposed to normal sunlight, there are usually 550–650 mm. of veins per square centimeter of lamina (fig. 42), though the veinage per unit area may vary to some extent in different portions of the leaf. Secondary xylem and to a lesser extent secondary external phloem are laid down in plants with a high carbohydrate content by an active cambium which extends from the axis out into the midrib for some distance in a leaf borne midway on the stalk (fig. 35, 39).[1] Annular, spiral, and rarely scalariform elements have been observed in the primary xylem of the midrib, and scalariform and pitted elements in the secondary xylem. The internal phloem has a greater abundance of sieve tubes and companion cells than does the external pholem, phloem parenchyma being more common in the latter.

Leaf thickness and proportion of tissues

The thickness of some 300 leaves, borne at various levels on the stalk, and grown under sun and shade conditions, averages 235 microns. The lamina is thickest just out from the midrib, about midway between the base and tip of the leaf. The thinnest portion is near the base. Thickness of the lamina may be correlated almost directly with the regions in which the greatest growth takes place, the more rapidly growing portions, in which the cells have not reached their final size, being thinner. The cuticle is thicker immediately adjacent to the midrib, and in general is thicker in older (distal) portions of the leaf. The proportion of spongy mesophyll to palisade is greater near the midrib, while the reverse is true toward the margins of the leaf. Stomata are abundant in both the upper and lower epidermis, being slightly more

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[1] An increased carbohydrate content may be induced in the upper leaves of the plant by removing the flower stalk soon after it first makes its appearance—the practice of "topping."
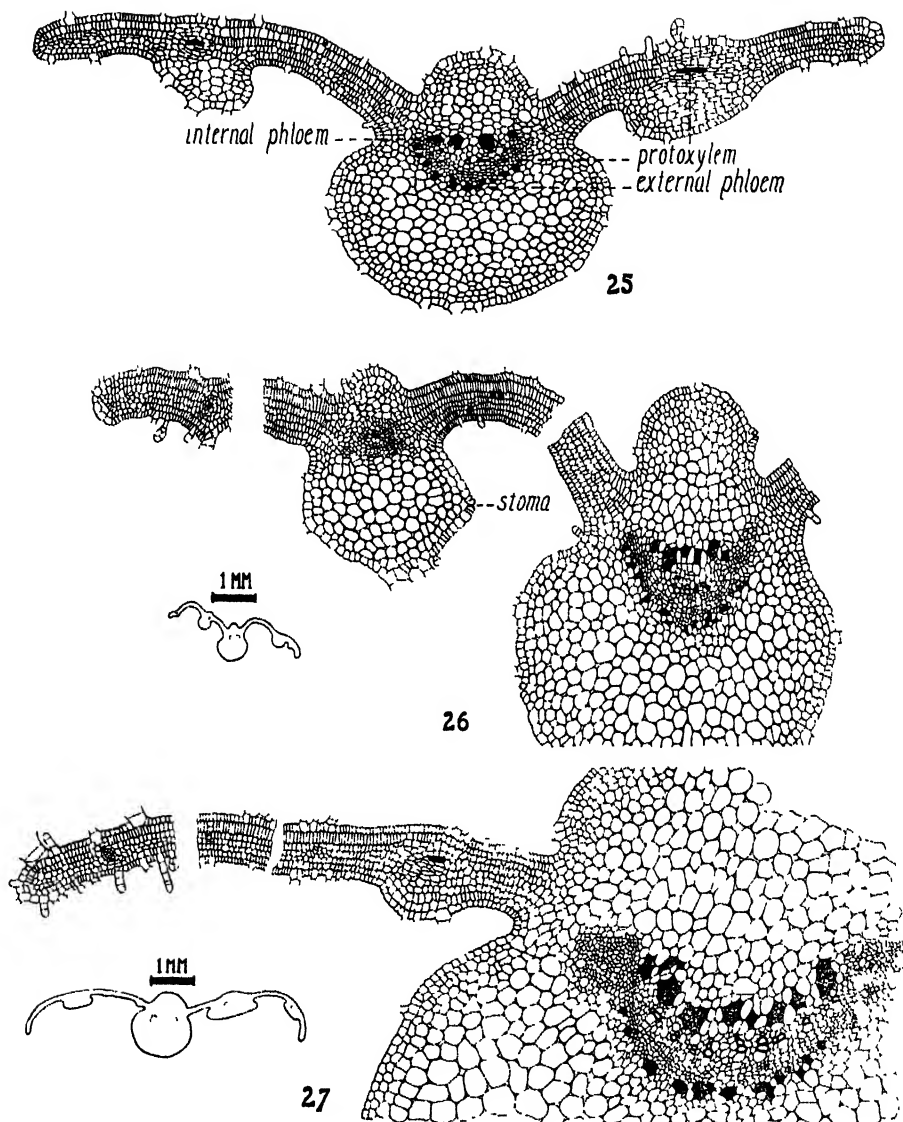
Fig. 25–27. Transverse sections of petiole and lamina, taken from midway between base and tip of successively older leaves. Fig. 25, primordium 8.5 mm. long (tenth leaf below growing point), showing first evidence of differentiation of xylem in the principal lateral veins (see fig. 21). Fig. 26, primordium approximately 15 mm. long, with well developed intercellular spaces, and stomata in the midrib and lateral vein. The final number of layers of cells of the mesophyll is established. Note embryonic portion of lamina just inside lateral vein. The millimeter scale applies only to the small diagram of the transverse section of the entire leaf through its widest portion. Fig. 27, primordium 24 mm. long (twelfth leaf below growing point). Note the pronounced increase in the number of protoxylem groups and internal phloem groups, as well as the general increase in cell size. The cuticle is developing on the epidermis of the midrib and lamina near it. The millimeter scale applies as above.

numerous in the lower. Epidermal cell size and the number of stomata per unit area depend upon several external factors, a discussion of which is not attempted here.

Relative growth of different portions of the lamina in proportion to the leaf as a whole

Figures 28 to 31 a represent different stages in the development of the same leaf, as described above. This leaf was marked off into 5-mm. squares as soon as it attained a workable size, and these small areas, hereafter designated as " segments," were measured at subsequent times (fig. 29–30) until maturity (fig. 31 a). The segments are so numbered in the diagram of the mature leaf (fig. 31 a) that the left hand side of the leaf is a mirror image of the right hand side. The segment numbers in 31 a apply also to the segments in corresponding positions in figures 28–30.
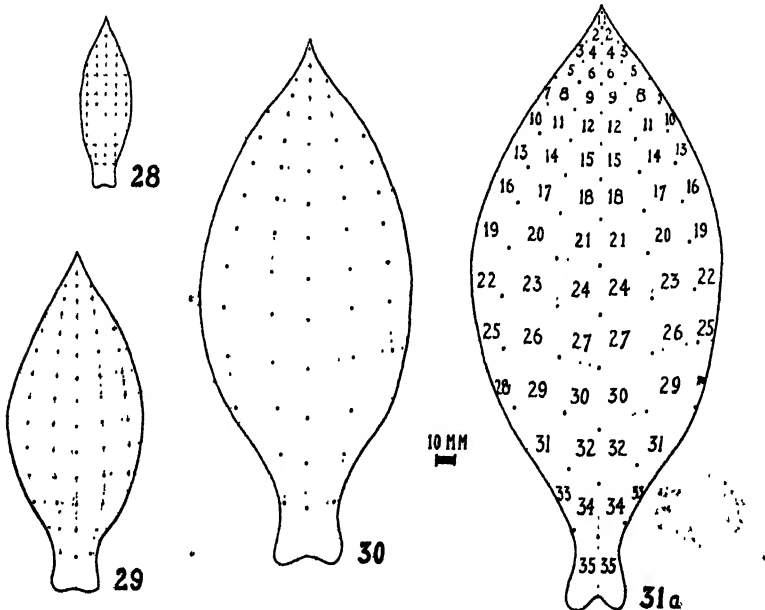
Fig. 28–31 a. Successive stages in the development of the leaf from the time it is ¼ its final size, to maturity. The entire leaf was marked into 5-mm. squares when 85 mm. long (fig. 28). The appearance of these segments as the leaf matures indicates the differing rates of expansion as the leaf grows to its final size. All numbered segments in fig. 31 a, for both right and left hand side of leaf, correspond to those in fig. 28–30. They also apply to those shown in fig. 31 b and 31 c.

It is obvious at a glance that the segments in the basal and central portions of the leaf have undergone a greater increase in size than have the segments toward the distal end—i.e., different portions of the leaf are growing at different rates. It is not so much the fact that any one segment has increased more

in area or in length in proportion to width than another segment, as it is the relative rate of growth of these segments to the growth of the leaf as a whole, hence the influence of these differential growth rates on the final shape attained by the leaf.

Where an organ is growing at a different rate from the organism as a whole, Huxley (1932) has suggested the term "heterogonic growth" and
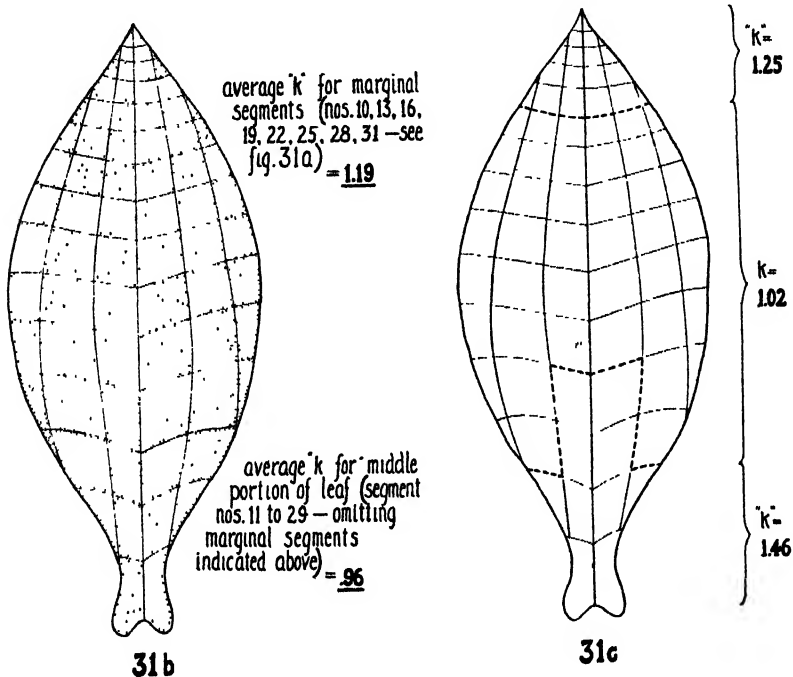
Fig. 31 *b* and 31 *c*. Fig. 31 *b*, diagram of the same leaf shown in fig. 31 *a*. The dots show the *average relative growth of the segments in area in proportion to the growth of the leaf as a whole* (see table 1 for data from which this diagram was constructed), and are thus roughly proportional to the number of cell divisions in the various segments. The basal and marginal segments show a greater relative increase in area (1.19 times greater) than the middle or apical segments, or than the leaf as a whole. Fig. 31 *c*, diagram to show *difference in rate of length and width growth in each segment*. The average " *k* " for length : width growth of segments in distal, central, and basal regions is indicated (see table 2 for data from which this diagram was constructed). This, together with 31 *b*, suggests that *localized* rather than *polarized* growth (except for the slight indication of polarized growth in the distal and basal regions) is primarily responsible for the shape of the leaf.

offers a formula which may be used to obtain a simple mathematical expression for such differences in relative growth rates. It is used in this study for obtaining quantitative expressions of differences in the growth rate of various portions of the leaf relative to the leaf as a whole, at different stages of development. It is as follows:

$y = b \ x^k$, which may be written log $y =$ log $b + k$ log x,

where $y =$ size of organ (used by Huxley for organism),

$x =$ segment of organ (used by Huxley for organ),

$b =$ value of y when $x =$ 1, and has no biological significance,

$k =$ ratio of growth rate of segment to growth rate of the leaf as a whole.

TABLE I. *Relative growth rates of segment area to leaf area, of the different segments at different stages in the development of the leaf. Stage I = fig. 28, stage II = fig. 29, stage III = fig. 30, stage IV = fig. 31 a. Figures under (a) refer to left hand side of leaf, (b) to right.*

Segment numbers 1–35 shown in figure 31 a apply also to the segments in corresponding positions in figures 28–30. The figures in the following table represent the values of " k " (Huxley), and are simply concise mathematical expressions to indicate the growth rate of a given segment in proportion to the growth rate of the entire leaf. If " k " (see text) for a given segment is less than 1.00, it indicates that that particular segment is growing in area less rapidly than is the leaf as a whole; if greater than 1.00, the segment is increasing in area more rapidly than the leaf as a whole. For graphic representation see fig. 31 b.

Segment no.	I–II (a)	I–II (b)	II–III (a)	II–III (b)	III–IV (a)	III–IV (b)	Average (a)	Average (b)	Average of (a) and (b)
1	.24	.73	.32	.03	.31	.45	.29	.40	.345
2	.23	.52	.35	.40	.31	.19	.29	.37	.33
3	.19	1.21	.52	1.07	.78	1.05	.50	1.11	.805
4	.37	.51	.46	.37	.92	.84	.59	.57	.58
5	.51	.53	.56	1.01	1.08	1.36	.72	.97	.845
6	.51	.59	.68	.54	.46	.88	.55	.67	.61
7	.55	.44	1.22	1.76	.22	.17	.66	.79	.725
8	.79	.82	.68	.69	.78	.57	.75	.69	.72
9	.77	.88	.76	.57	.42	.59	.65	.68	.665
10	.93	.83	.91	.58	1.18	1.60	1.00	1.00	1.00
11	.91	.88	.69	.87	.81	.62	.80	.79	.795
12	.85	.94	.74	.78	.81	.55	.80	.76	.78
13	1.00	1.00	1.05	.68	.94	1.07	1.00	.92	.96
14	1.10	1.00	.75	.95	.42	.45	.76	.80	.78
15	.99	1.09	.91	.86	.51	.53	.80	.83	.815
16	1.17	1.21	1.00	.63	.94	2.95	1.04	1.60	1.32
17	1.15	1.06	.35	.98	1.02	.78	.84	.94	.89
18	1.01	1.11	1.00	1.01	.97	.81	.99	.98	.985
19	1.43	1.34	.67	.79	1.60	1.47	1.23	1.20	1.215
20	1.24	1.19	1.07	1.03	.71	.53	1.01	.88	.945
21	1.18	1.19	1.01	1.14	.98	.57	1.06	.97	1.025
22	1.48	1.45	.78	1.18	1.71	1.39	1.33	1.34	1.335
23	1.24	1.24	.68	.94	2.25	.95	1.39	1.04	1.215
24	1.18	1.26	1.06	1.09	1.04	1.07	1.09	1.14	1.115
25	1.47	2.15	1.20	1.65	.59	.70	1.09	1.50	1.295
26	1.31	1.29	1.30	1.14	.65	.73	1.09	1.05	1.07
27	1.16	1.31	1.22	1.32	.78	.24	1.05	.95	1.00
28	1.33		1.51	1.73	1.06	1.32	1.30	1.52	1.41
29	1.23	1.32	1.40	1.39	1.00	.76	1.21	1.16	1.185
30	1.08	1.36	.83	1.23	2.14	1.05	1.35	1.21	1.28
31	1.17	1.27	1.26	1.17	1.82	2.97	1.08	1.80	1.44
32	.98	1.66	1.24	1.35	.88	.96	1.03	1.32	1.175
33	1.02	1.84	1.10	1.20	2.13		1.09	1.52	1.305
34	1.41	.93	1.12	1.57	1.20	.32	1.24	.94	1.09
35	.76	.83	.78	.95	.40		.65	.89	.77

The value of k, for example, if determined for the increase in area of a segment with regard to the increase in area of the leaf as a whole, would represent a simple quantitative measure of the growth of that segment in proportion to the growth of the entire leaf. If $k = 1$, for a particular segment, it would indicate that that segment was increasing at the same rate as the leaf as a whole, or if less than 1, that the segment was growing at a slower rate than the leaf as a whole, etc. Such values for k have been determined (table 1). Figures are given for corresponding segments on the right and left hand sides of the leaf. The average k for each segment for the three stages of growth is also given for each side of the leaf, (a) and (b), and the average of both sides. It will be noted that in many cases the rate of increase of size for a given segment is greater in the early stages of growth, and decreases toward maturity (seg. 27). In other cases it is just

TABLE 2. *Relative growth rates of length and width in each segment as indicated by values of "k" at different stages of development. All stages and segment numbers correspond to those used in table 1. Where "k" is greater than 1, a more rapid growth rate for length is indicated; where it is less than 1, for width. For graphic representation see fig. 31 c.*

Segment no.	I–II (a)	(b)	II–III (a)	(b)	III–IV (a)	(b)	Average (a)	(b)	Average of (a) and (b)
1	1.49	1.24			1.77		1.63	1.24	1.546
2	1.37	.83	1.75	1.56	2.55		1.89	1.20	1.545
3	.83	2.71	2.38	.32	.85		1.35	1.51	1.43
4	1.75	.71	.65	1.65	1.46	1.54	1.28	1.30	1.29
5	1.39	.54	.89	.88	.67	1.96	.98	1.13	1.055
6	1.00	.57	1.00	1.98	1.95	1.10	1.31	1.22	1.265
7			1.11		1.16		1.13		1.13
8	1.05	1.18	1.18	.80	.62		.95	.99	.97
9	1.32	1.00	.68	.85	1.17	1.53	1.05	1.13	1.09
10	1.10	2.24	.40	.71	1.13	.26	.88	1.07	.975
11	.93	1.00	1.04	.83	.94	2.92	.97	1.58	1.275
12	1.08	.88	.77	.80	1.57	1.74	1.14	1.14	1.14
13	1.26	1.00	.63	1.42	.33	.37	.74	.93	.835
14	.98	1.00	1.23	.86	.52	.72	.91	.86	.885
15	1.24	.95	.80	.81	.64	1.12	.89	.96	.925
16	.83	.85	.85	1.15	.46	.31	.71	.77	.74
17	.85	.91	1.17	.82	.27	3.90	.76	1.88	1.32
18	1.00	.83	.92	.85	1.07	2.34	1.00	1.34	1.17
19	.73	.71	1.90	2.30	.57	.06	1.07	1.02	1.045
20	.99	.96	.82	1.03	1.60	.78	1.14	.92	1.03
21	1.10	.96	.99	.93	.67	.66	.92	.85	.885
22	.70	.82	1.78	.87	.68	1.00	1.05	.89	.97
23	.96	.96	.80	1.03	1.37	1.18	1.04	1.06	1.05
24	1.06	.93	1.01	.87	.90	1.14	.99	.98	.985
25	.80	1.10	1.51	.88	.24	.52	.85	.83	.84
26	1.00	1.04	.97	1.35	.30	.60	.76	1.00	.88
27	1.21	.87	1.17	1.29	.48	.57	.95	.91	.93
28	1.14		.79	.74	.70	.44	.88	.59	.735
29	1.01	1.12	1.15	1.12	.63	.40	.93	.88	.905
30	1.27	1.00	1.30	1.23	1.16	1.18	1.24	1.13	1.185
31	1.07	1.03	.91	.78	.56	1.10	.85	.97	.91
32	1.62	1.09	1.16	.91	2.32	2.51	1.70	1.50	1.60
33	1.32	1.63	4.11	2.55	.69		2.04	2.09	2.065
34	1.78	1.49	1.69	.94	1.49		1.65	1.21	1.43
35	.71	.66	2.52	.87	.56		1.26	.77	1.015

the opposite (seg. 10), and in still other cases the increase continues in about the same proportion to that of the leaf as a whole (seg. 24). These differences in the distribution of growth, or the differential rates of growth, are responsible in part for the final shape attained by the leaf, and emphasize the importance of *localized growth* in the development of the shape of an organ.

The data as regards increase in area bear out the conclusions from a study of tissue structure, namely, that the apical portion of the leaf matures early. In segment 1 (both sides), for example, the increase in size takes place only ⅓ as rapidly as the leaf as a whole. Segment 18 (both sides), on the other hand, increases at approximately the same rate as the leaf as a whole. By using segment 18 where k is approximately 1, the average relative growth rates of different portions of the leaf may be seen graphically (fig. 31 b). Those segments with a greater density of dots than segment 18 have undergone a greater relative increase in size than the leaf as a whole (have retained their meristematic character for a longer time), while those having a lesser density of dots than segment 18 have undergone a relatively slower growth (differentiate earlier) than the leaf as a whole. The varying densities of dots bring out equally strikingly the gradient down the leaf from tip to base (table 3), as well as the gradient across the leaf. Table 3 and figure 31 b show the marginal segments, midway between base and tip, increasing in width at a markedly greater rate than the leaf as a whole.

Figure 31 c attempts to show graphically what is set forth in more detail in table 2—i.e., the growth rates of length and width in each segment as indi-

TABLE 3. *The axial gradient. Relative growth of linear units along the midrib compared with increase in the length of the leaf as a whole. The numbers applied to segments on either side of the midrib in tables 1 and 2 apply in this table to the linear units along the midrib from the leaf tip to segment 34. The figures represent the values of " k."*

The relative rate of length-growth of units in proportion to the leaf as a whole increases from 1–30, then decreases, but even unit 34 is increasing in length approximately 1.24 times (average) as rapidly as the leaf as a whole.

All stages and segment numbers correspond to those used in tables 1 and 2.

Linear unit no.	I–II	II–III	III–IV	Average
1	.24	.66	.00	.30
2	.24	.51	.48	.41
4	.62	.27	.78	.56
6	.62	.51	.36	.50
9	.92	.60	.81	.78
12	.92	.69	1.02	.88
15	1.10	.90	.42	.81
18	1.10	.90	1.20	1.07
21	1.26	1.05	1.01	1.11
24	1.31	1.02	1.29	1.21
27	1.26	1.46	.72	1.15
30	1.31	1.42	1.71	1.48
32	1.26	1.31	1.19	1.25
34	1.31	1.23	1.19	1.24
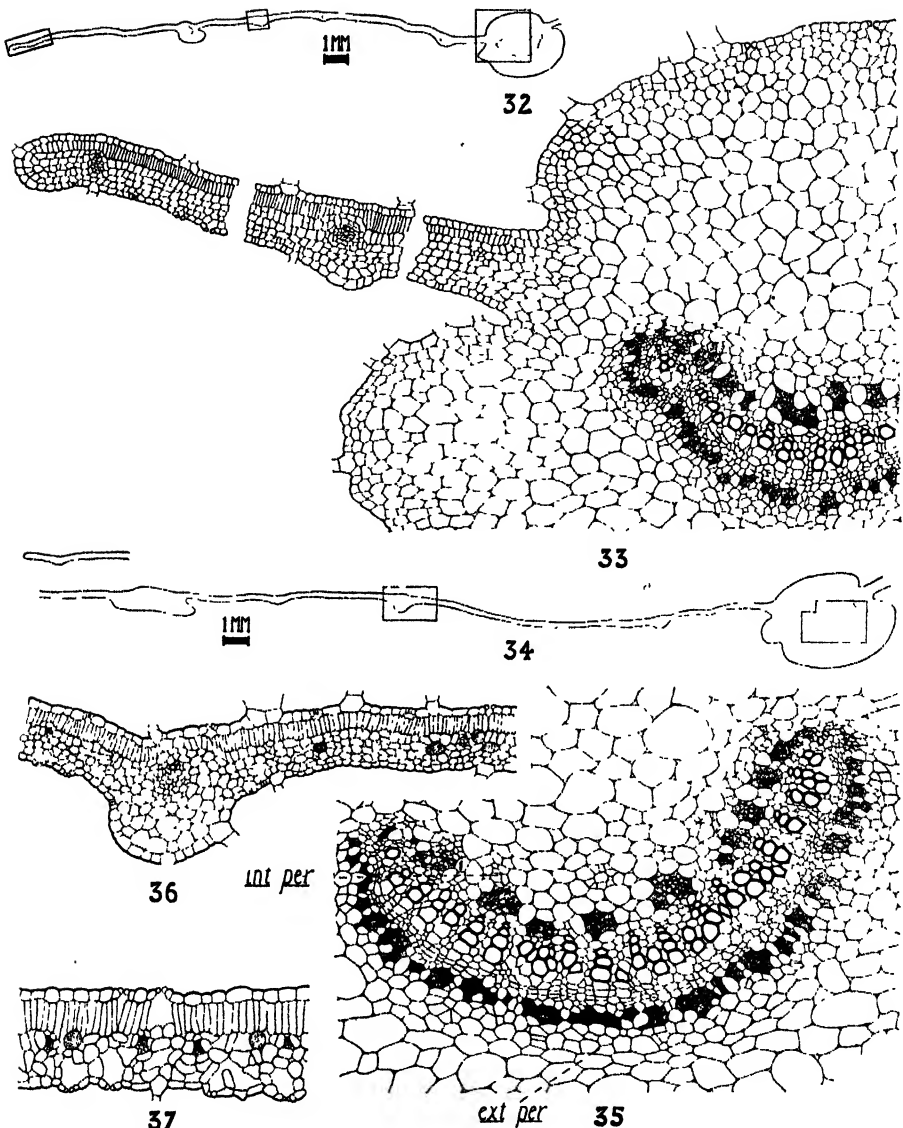
Fig. 32-37. Fig. 32, diagram of transverse section, with millimeter scale, of midrib and portion of the lamina taken from midway between base and tip of leaf 135 mm. long (halfway in size between those shown in fig. 28 and 29). Fig. 33, detailed drawings of portions indicated in fig. 32. Note the appearance of stomata in the lower epidermis, the marked increase in cell size in all regions, and the appearance of a cuticle in certain portions. Fig. 34, similar to fig. 32, of leaf 150 mm. long (approximate size of that shown in fig. 29). Fig. 35 and 36, detailed drawings of portions indicated in fig. 34. Both internal (*int per*) and external pericycle (*ext per*) are beginning to differentiate in the midrib bundle. In the lamina it is possible to observe large intercellular spaces, stomata in both upper and lower epidermis, a conspicuous cuticle, and cells bearing crystals of calcium oxalate. Fig. 37 shows a transverse section of a small portion of the lamina of a leaf 210 mm. long (approximately halfway in size between those shown in fig. 29 and 30—2/3 mature size).

Gradient across leaf. Relative growth of middle tiers of segments in width compared with increase in width of the leaf as a whole. The common boundary between segments 19–22, 20–23, and 21–24 is used for segment width. The figures represent the values of " k." Note that the marginal segments have increased in width 1.36 times as rapidly (on the average) as the leaf as a whole, while the segments nearer the midrib have increased approximately in proportion to the width of the leaf as a whole (within the limits of error).

All stages and segment numbers correspond to those used in tables 1 and 2.

Width unit no.	I–II		II–III		III–IV		Average		Average of
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a) and (b)
19–22	1.37	1.30	.52	.86	1.86	1.89	1.37	1.35	1.36
20–23	1.02	1.01	1.25	.94	.52	.67	.93	.87	.90
21–24	.92	1.02	1.01	1.14	1.03	.77	.98	.98	.98

cated by values of k at different stages of development.   It is clear that the increase in each dimension is about equal throughout the large central portion of the leaf, while some of the distal and basal segments show a greater increase in length in proportion to width.   These differences in the distribution of *polarized growth,* that is, greater growth in one dimension than in another, together with the differences in *localized growth* pointed out above, are responsible for the final shape attained by the leaf.

Relative growth rates of length and width for the leaf as a whole indicate that it increases in width only eight-tenths as rapidly as in length.

DISCUSSION

In the present study emphasis has been on the development of the leaf rather than on its ultimate ontogenetic origin.   With regard to the latter, however, most investigators (Lamounette, 1890; Douliot, 1890, 1891; Koch, 1893; Flot, 1905; Herrig, 1914; Schmidt, 1924; Lange, 1927; and others) agree that the embryonic epidermis of the stem (dermatogen) gives rise to that of the leaf, and the embryonic cortex (periblem) gives rise to the internal tissues.   The evidence from a brief study of the growing point of the tobacco stem supports such a contention, but the two internal primary meristems are not clearly distinguishable at the time leaf development is initiated.

That the midrib is the first portion of the primordium to arise as a definite outgrowth of the axis, and that the lamina arises from it soon after, are facts which seem more important here than the ultimate ontogenetic origin of the leaf.   As regards the initial stages of lamina development, Schwarz (1927) reports that in *Plectranthus* the first indication of lamina formation is a doubling of the subepidermal layer along the sides of the primordium (marginal growth) when it is about 40 microns in length.   It has been shown above that this does not take place in tobacco until the primordium is approximately 600 microns in length, the leaf of the latter being much larger at maturity than that of *Plectranthus*.   Marginal growth was also observed in a general way by Nägeli (1846) and Prantl (1883).

1MM

38

internal endodermis

" pericycle (thin-walled)

" " (thick-walled)

" phloem

protoxylem

} primary

metaxylem

secondary xylem

secondary phloem

primary phloem

external pericycle

" endodermis

39

upper epidermis

palisade — upper mesophyll

-for-
origin of
tissues
-see figs.
13-17.

middle mesophyll

} spongy
mesophyll

lower mesophyll

lower epidermis

stoma

xylem
phloem

} vascular
bundle

40

Fig. 38-40. Fig. 38, diagram of transverse section of midrib and portion of the lamina, of a mature leaf, natural size (leaf size in tobacco varies so much in the different varieties and when grown under different conditions that the sizes given here should be considered only in relation to the more detailed studies presented in connection with them). Fig. 39, detailed drawing of portion of vascular arc indicated in fig. 38. Internal and external endodermis are discernible, and the walls of certain cells of the internal and external pericycle are considerably thickened. Note the large amount of secondary xylem as contrasted with the small amount of secondary phloem. The cambium (see footnote 1) ceased activity sometime before the leaf was harvested. Fig. 40, transverse section of portion of the mature lamina.

The development of the tobacco leaf is in many respects similar to that of *Pelargonium,* described by Nöack (1922). He finds the apex of the primordium as well as the margin of the young lamina adding cells by a " doubling " of the subepidermal layer at the tip and margins respectively. In tobacco, it is as if there were a subepidermal cell to initiate the formation of the primordium and carry it along as would an apical cell (fig. 17) until 2 to 3 mm. in length, at which time the apex matures (the leaf being 1/30 or less its final size). Steinheil (1837) and Nägeli (1846) were among the first to report similarly—i.e., that certain leaf primordia possess in the beginning an actively growing tip, which often is the first portion of the leaf to mature. Sonntag (1887) has shown similarly that the leaf apex of *Geranium* matures when the primordium is approximately 1.75 mm. long. He gives figures for other leaves also. Račiborski (1900) has likewise demonstrated that the tip of the *Benincasa* leaf is mature when the primordium is approximately 1 mm. long, or about ⅛ its final size.

In the present study such specificity exists as regards the origin and continuation of cell layers in the lamina that the work of Gravis (1907) and Flot (1905) might well be recalled here. From studies of *Tradescantia* and other forms they reported the existence of three generative layers in the leaf primordium itself. These they interpreted as corresponding to the three primary histogens of the stem. This conception was new at that time. Their results may be outlined as follows:

(1) epidermal meristem ⎰upper or " internal "⎱ 1st histogen
　　　　　　　　　　　 ⎱epidermis　　　　　　⎰

(2) cortical meristem ⎰upper, or " cortical "⎱
　　　　　　　　　　　⎨layer of mesophyll　⎬ 2d histogen
　　　　　　　　　　　⎩(palisade)　　　　　⎭

(3) vascular meristem ⎧foliar " pith "　　　　　　　　　　　　⎫
　　　　　　　　　　　⎪xylem　　　　　veins and middle ⎪ 3d histogen
　　　　　　　　　　　⎨phloem　　　　　mesophyll　　　 ⎬
　　　　　　　　　　　⎩foliar pericycle　　　　　　　　　⎭

(2) cortical meristem ⎰lower " cortical " tissue　⎱ 2d histogen
　　　　　　　　　　　⎱or lower layer of mesophyll⎰

(1) epidermal meristem ⎰lower, or " external "⎱ 1st histogen
　　　　　　　　　　　⎱epidermis　　　　　　⎰

This scheme to indicate the origin of cells in each layer of mesophyll is too similar to the results of Nöack for *Pelargonium* and figures 15 and 16 in this paper to escape attention. Schwarz, however, finds that in *Plectranthus* the four layers of mesophyll are formed as a result of tangential division of the lower subepidermal layer. Schüepp (1926, p. 4), agreeing essentially with Gravis and Flot, recognizes the three primary meristems in dicotyledonous leaves, and gives them the more commonly accepted names: protoderm, procambium, and ground meristem. They correspond to the epidermal, vascular, and cortical meristem, respectively, of Gravis and Flot.

With regard to increase in the surface of the lamina, Schüepp's (1926, p. 18) description of the " Plattenmeristem " is among the most important contributions to our knowledge of leaf development.   Such a *plate meristem* consists of all parts of the lamina which are increasing their surface area, the meristematic lamina giving rise to additional lamina, etc.   He interprets the large expansion as due to surface growth " which without variation in the number of cell layers and almost without any thickening growth " . . . increases its surface many times over.   In such a plate meristem, he distinguishes two sharply defined directions of cell division: (1) the direction perpendicular to the surface (divisions in this plane usually take place near the margin of the tobacco lamina and continue only until the characteristic number of layers is established), and (2) the direction parallel to the surface, the latter making possible the marked expansion in one plane.   He further points out the stratified appearance of cell layers in a transverse section of the leaf, but notes that a section cut parallel to the plane of the lamina shows a complete lack of arrangement of cells—i.e., the nuclear spindles are oriented in any direction.[2]   It is clear, then, that in the growth of a plate meristem, each mesophyll mother cell by a succession of divisions gives rise to a small " plate " of cells.   These plates are irregularly shaped discs one cell in thickness and a few cells across in surface view.   Schüepp suggests that the whole plate meristem is little more than an association of these irregularly shaped discs, congenitally grown together.

From the foregoing discussion of the plate meristem, and in the light of the work of Gravis, Flot, Nöack, and Schwarz, together with the evidence given here, a relative constancy in the number of cell layers of mesophyll in leaves might be expected.   Famintzin (1875) reports the lamina of *Phaseolus multiflorus* as consisting of 6 morphologically different layers, and that any greater numbers in the mature leaf result from the division of existing ones. Similar results, either figured or described, have appeared in the works of Nöack for *Pelargonium,* Mounts (1932) for *Vitis* and *Catalpa,* Hayward (1932) for *Ipomoea,* Jensen (1933) for *Nicotiana paniculata,* and there have been innumerable other contributions.   While some of the younger leaves of tobacco show 6 layers (fig. 25), others show 7 when the lamina is clearly established (fig. 23 b).   Not more than 7 layers have been found in leaves of any degree of maturity (fig. 36, 37, 40), and it has been pointed out that the young lamina on one side of the midrib may differ in size and thickness from that on the other side.   The average thickness of the lamina, 235 microns, agrees closely with the figure of 230 microns given by Dose (1914, p. 62).

With regard to internal phloem, the findings here are generally in agreement with those of other investigators.   Lamounette (p. 272) has observed that in the midrib of the tobacco leaf, the external phloem is made up of a large number of small sieve tubes, separated from xylem by active cambium,

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[2] Such an orientation of nuclear spindles confirms the interpretation of the lamina increasing its area largely by localized growth, as brought out above.

while the internal phloem is composed of less numerous, but better developed sieve tubes.   Some of the internal phloem groups are separated from the first thickened xylem element by one or two parenchyma cells, others by 5 or 6. The later differentiation of the internal phloem groups has been noted by most
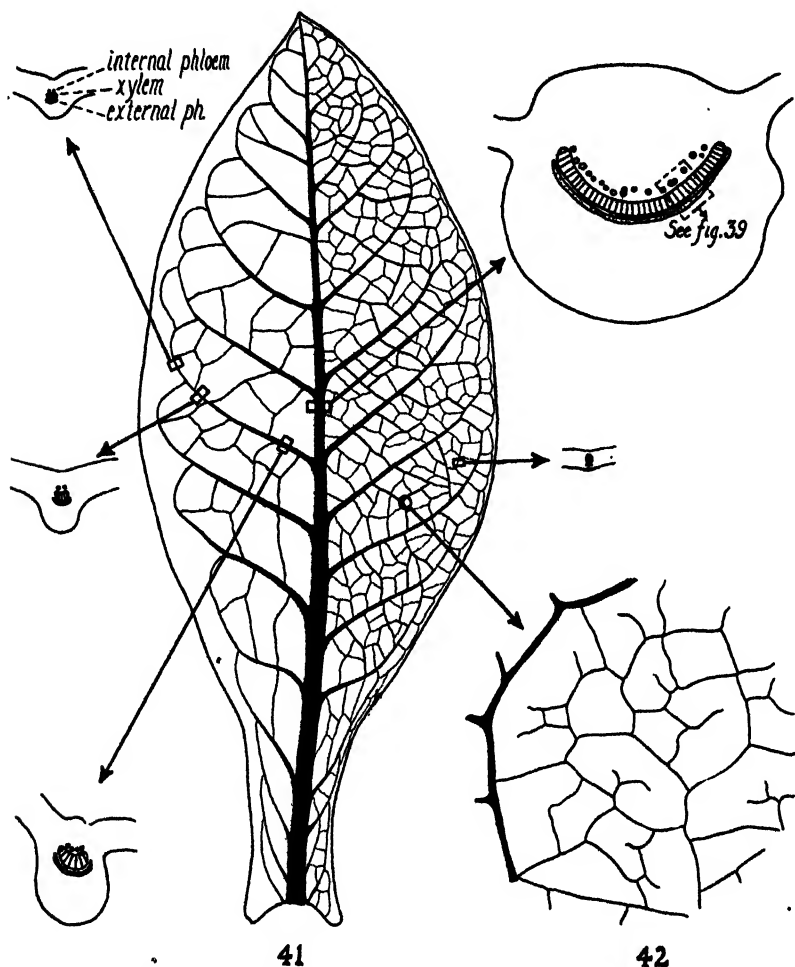
41   42

Fig. 41–42. Fig. 41, diagram of mature leaf (1/3 natural size), showing midrib, principal lateral veins, and the coarser vascular network. The internal phloem is found only in the midrib and principal lateral veins, as shown in the small diagrams of transverse sections from various places in the leaf. Fig. 42, a small portion of the lamina, showing the ultimate network of veins (× 20). This leaf came from midway on the stalk, and there were 543 millimeters of veins per square centimeter of lamina.

workers from the time of Herail (1885) to the present. The fact that the internal phloem is definitely separated from the xylem, coupled with its later maturity, has been interpreted by these two authors as indicating a morphological difference in the origin of the two tissues. The evidence in this study

agrees with that just cited, though the interpretation differs. With the loss of the endodermis as a definite morphological entity in the leaf, it is impossible to interpret with precision. But the presence of starch grains in a single layer of cells around the midrib bundle just outside the external pericycle, and immediately surrounding the internal pericycle (suggesting its endodermal nature—fig. 39), strongly suggests that all tissues within originate from pro-vascular meristem. The distribution of internal phloem in the veins of *Ipomoea* is reported by Hayward. He finds it present in the median and two principal lateral veins. The other two lateral veins are transitional. This is in marked contrast to the distribution in the midrib and all principal lateral veins of the tobacco leaf.

De Toni and Paoletti (1891) report " secondary elements entirely lacking " in the tobacco leaf. Although this is usually the case, any large accumulation of carbohydrates in the plant stimulates cambial activity in the midrib.

As regards the possibility of tissue strains and related phenomena, Lee and Priestley (1924) state that the cuticle as it condenses is resistant to stretching, therefore possibly affecting the subsequent direction of stretching of parenchymatous tissue, such as the palisade of the mesophyll. This is in close accord with Pfeffer (1903, p. 45): " That the cuticle is under strain is shown by the fact that the outward curvature of epidermal strips from *Agave* and *Hyacinthus* is produced in water. . . ." It has been shown here also that a slight tension exists between the mesophyll and the epidermal layers when the leaf is quite young. While it is problematical whether this is due entirely to resistance of the cuticle to stretching, or to some extent to a lagging behind in the division and enlargement of epidermal cells, nevertheless, it does seem clear that the mesophyll takes the initiative in developing the form of the young leaf—the epidermis simply behaving like an independent layer of a periclinal chimera. The extended growth period of the tobacco leaf, in which the epidermis takes the leading part in at least the last half of the period of expansion, certainly does not support the contention of Lee and Priestley with regard to the cuticle and its resistance to stretching.

It has been shown that cell division ceases first in the epidermal layers during leaf ontogeny, and that this cessation is followed closely by that of the spongy cells, then the palisade. It is clear also that the epidermal cells continue their enlargement (in the plane of the leaf surface) over a longer period of time than do any of the mesophyll cells. This explains why cells of the spongy tissue, having ceased dividing and apparently lacking the ability to continue enlargement, are literally pulled apart. Pfeffer observes that " the union between component cells in a growing tissue is frequently partially or entirely dissolved, as for example during the formation of intercellular spaces . . . in leaves. This is attained by the partial or complete conversion of the middle lamella of the common partition wall into substances which imbibe water, and swell or dissolve, so that a slight strain suffices to cause separation between the contiguous layers in question."

The strains resulting from continued enlargement of epidermal cells are approximately equal in all directions in the plane of the surface, resulting in a pulling apart of the cells in such a way that a uniform spongy condition develops. That the spongy mesophyll cells are not without their reciprocal effect on the lower epidermis is also clear. They offer a definite resistance as they are pulled this way and that by the adjacent enlarging epidermis, so that distortions and waviness of lateral walls of the lower epidermal cells might therefore reasonably be expected to develop. If enlargement of the leaf continues long enough, the palisade cells are also pulled apart, even though they continue dividing longer than any other cells of the leaf, and they too in offering random resistance bring about waviness in the lateral walls of the upper epidermal cells. This waviness is seldom as pronounced as that in the lower epidermis (figures shown by Schramm, 1912, and numerous others).

In the light of the above discussion of internal structure as it comes into play in leaf ontogeny, it now seems appropriate to include the scheme sug-gested by Schüepp (1918) to show the derivation of the individual parts in the gross development of the leaf, from the primordium to maturity:

ventral lobe (lower part of leaf primordium) ——————— ————→ *leaf sheath*
    (procambium, semimeristem)
        ↗
leaf primordium ——————— ——————→ *petiole* ————— ————— ————→ *petiole*
    (meristem)
        ↘
    epiphyll ——————— — — ——————→ *principal veins* ————— —————→ *principal veins*
        (meristem)   ·   (procambium, semimeristem)
            ↘
    primordial lamina
        (meristematic lines of growth, i.e., narrow zones of meristematic activity)
                ↘
        lamina ——————————→ *lateral veins* ———————————→ *lateral veins*
            (meristem)   (procambium, semimeristem)
                    ↘
            lamina ————————→ *secondary veins* ————————→ *secondary veins*
                (meristem)
                        ↘
                lamina ————→ *procambial strands* ————→ *vascular bundle*
                    (meristem)   *network*

→ *mesophyll*

Interpreted in terms of the tobacco leaf, it merely indicates that the "leaf primordium" (the short embryonic midrib) develops an upper portion which in turn gives rise to the lamina, veins, etc., and to such petiole as may exist. Its lower portion or "ventral lobe" might be considered as giving rise to the partially decurrent and half-clasping basal end of the leaf, so characteristic of most varieties of *Nicotiana tabacum*.

Some of the older studies on the laying down of leaf surface, regions of growth, etc., are of less importance as a result of Huxley's recent contributions

to the study of relative growth, but any consideration of leaf development would be incomplete without them. The works of Nägeli and Prantl were especially important because they distinguished between the rôles of increase in cell number and subsequent cell enlargement in the expansion of the lamina. The former (p. 162, etc.) distinguished 3 types of growth in leaves: (1) certain compound leaves such as *Utricularia* in which cell division and subsequent enlargement continues to take place at the tips and along the periphery after growth in the basal region has stopped; (2) those leaves in which cell division stops throughout the whole leaf at approximately the same time, followed by expansion throughout; (3) those leaves in which peripheral cell divisions cease first in the upper part of the leaf, but continue along toward the base and cease last at the base, the tip being the first portion to mature in this type. Prantl has extended Nägeli's work in an attempt to classify the leaves of plants from many families according to the location of the meristem in the laying down of their leaf surface. He too has distinguished 3 types: (1) Those in which the meristem is active in the beginning at the tip of the primordium, proceeding toward the base, and remaining active at the base and (at the same time) in a transverse direction until cell division is completed. Enlargement proceeds basipetally and transversely at the same time, but takes place principally in a lengthwise direction. Simple leaves with prominent midribs, such as *Salix, Celtis,* etc., characterize this type (" basiplastic," p. 281). (2) Those of the above type in which the midvein differentiates early (thereby " forcing " the meristem to the margin) mark the transition to a second type which is characterized by the marginal position of the meristem. The whole leaf is meristematic for a time, and except for the margin, which continues to be meristematic, it goes from this condition over entirely into one of enlargement. Among simple leaves of this type, *Cercis, Syringa,* and *Rhamnus* are mentioned (" pleuroplastic," p. 284). (3) In still another type lobes develop while the entire primordium is uniformly embryonic, as in *Malva borealis,* or in the case of compound leaves the leaflets may arise in acropetal succession as in *Juglans, Robinia,* etc. The individual leaflets may develop according to the first two types (" eucladous," p. 284, 285). Goebel (1905, p. 312) states: " A sharp limit is not to be drawn between these types, especially between the first and second, and the advantage of the grouping . . . appears very doubtful." However, the distribution of growth during the ontogeny of the tobacco leaf is such that the third type distinguished by Nägeli describes the developmental condition rather well, as does Prantl's " pleuroplastic " type.

Huxley's more recent work has opened new possibilities in the heretofore loose consideration of regions of growth in different organs. It has become possible to analyze with exactness the relative rates of growth in any part of an organ, thus obviating the purely qualitative nature of such work as that of Nägeli, Prantl, and still others more recent. In the case of the tobacco leaf, the determinations of " k " have given a clear quantitative expression of the distribution of growth in its different portions. It has become pos-

sible to put growth in terms of simple figures which make it possible to analyze the development of form, a fact which should be of increasing importance to geneticists and morphologists.

SUMMARY

1. The leaf arises as a lateral projection of the embryonic stem tip. Its initial impetus for development comes from a few localized dividing and enlarging cells in the outer layers of the promeristem, in the portion which becomes periblem, and still later, cortex. The origin of tissues in the leaf is discussed.

2. The leaf primordium becomes the center of a new direction of growth. It possesses a subepidermal cell at its tip which behaves as an apical cell until the young leaf is 2–3 mm. long; the tip then matures. Subsequent divisions of cells laid down by this apical cell are responsible for the beginning of midrib development.

3. The midrib primordium has no lamina until approximately 0.6 mm. long, when the lamina is initiated by the activity of a row of subepidermal cells on each side of the midrib primordium, in effect, a marginal meristem. All cells laid down by it are mesophyll mother cells. They undergo subsequent divisions throughout the lamina for some time. The epidermis is self-perpetuating.

4. The upper (palisade) and lower layers of mesophyll, once established by the marginal meristem, perpetuate themselves in a plane parallel to the leaf surface. The middle mesophyll (spongy region), ordinarily three layers of cells in thickness, behaves likewise.

5. Cell divisions cease first in the epidermis, followed by the middle and lower mesophyll, then the palisade. The middle mesophyll remains potentially provascular, and all lateral veins, large or small, differentiate from it. They may develop long after cell division has ceased in the remainder of the leaf. The fact remains, however, that division ceases in this and the lower mesophyll regions as a whole, immediately after it ceases in the epidermal layers.

6. Although it is the epidermis, both upper and lower, in which a cessation of cell division first takes place, epidermal cell enlargement continues longer than cell enlargement in any other tissue. It is the strain of the expanding epidermis on cells of the middle and lower mesophyll, together with the fact that these mesophyll cells cease dividing and their rate of enlargement is insufficient to meet the strain, that causes them to pull apart, and the uniform spongy condition to result.

7. The reciprocal stresses of the developing spongy tissue on cells of the lower epidermis cause its lateral walls to become distorted and wavy. A similar relationship exists between the upper epidermis and the palisade layer, but to a lesser degree.

8. Palisade cells begin to acquire their characteristic shape when the leaf is 4 to 5 mm. long (1/75 to 1/60 final size), although they continue to divide for a time after this. Intercellular spaces (and their associated spongy

parenchyma) do not develop markedly until the leaf is 80 to 100 mm. long (¼ to ⅓ final size).

9. The xylem and external phloem begin to differentiate in the midrib when the primordium is approximately 1 mm. in length. The internal phloem is not apparent until the primordium is over twice this length. The principal lateral veins do not show differentiated vascular tissue until the leaf is 8.5 mm. long. Internal phloem is confined to the midrib and principal lateral veins. The cambium is active in the midribs of leaves of plants high in carbohydrates.

10. Three hundred leaves, from all levels on the stalk, and grown under diverse conditions, average 235 microns in thickness.

11. Huxley's formula for obtaining values for "*k*" has been used to obtain quantitative expressions for growth in the different portions of the leaf (relative growth). On the whole there is greater growth in area in the marginal, central, and basal portions of the lamina.

The final shape attained by the leaf as a whole is due in part to a differential distribution of growth in its various portions—i.e., *localized growth*, and also to differences in the distribution of *polarized growth*—that is, greater growth in one dimension than in another in various portions of the leaf. Together they are responsible for the shape of the organ. Relative growth rates for the leaf as a whole indicate that it increases in width only 0.8 as rapidly as in length.

Huxley's formula also makes possible the determination of a linear growth gradient along the midrib, from the early-maturing leaf-tip to the relatively late-maturing base. A similar gradient across the leaf shows the marginal segments as having increased in width more rapidly than those nearer the midrib.

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FREEZING INJURY TO ARBORVITAE AND JUNIPERS IN KANSAS [1]

L. E. MELCHERS

The susceptibility of Arborvitae and Junipers to freezing injury in Kansas was shown in the spring of 1932. It seems possible that similar injury to the species of evergreens named herein could result in neighboring states. The differences in injury were so striking that it was plainly evident that certain varieties were much hardier than others. Landscape and amateur gardeners could well afford to give the matter of planting evergreens careful consideration.

The month of March, 1932, averaged the sixth coldest March on record in Kansas and was 3.5° F. colder than the February preceding. The mean temperature for March, 1932, was 36.9°, which is 6.7° below normal, making it the coldest March in eight years. The week beginning with the 5th of March was the coldest week in March in the history of the state, with temperatures scarcely up to the freezing point on any afternoon and readings close to or below zero on most of the nights. The temperatures and precipitation records from February 15 to March 15, 1932, are given for Manhattan, Kansas. The maximum and minimum temperatures for the first two weeks in February are not included in the accompanying figures since they are very similar to the last two weeks.

In order to appreciate the situation, one should examine the temperature records preceding the severe March freeze. The maximum daily tempera-

Temperature and precipitation records, February 15 to March 15, 1932, Manhattan, Kansas

		Degrees F. Max.	Min.	Precipitation Inches			Degrees F. Max.	Min.	Precipitation Inches
February	15	33	27		March	1	65	43	.06
	16	59	31	.55		2	57	35	
	17	33	18			3	49	38	
	18	43	23			4	40	20	.25
	19	45	23			5	23	5	
	20	40	25	.10		6	18	6	.04
	21	53	36			7	15	-3	
	22	49	28			8	14	5	
	23	58	28			9	18	6	
	24	68	28			10	22	0	
	25	73	36			11	25	7	
	26	72	33			12	27	8	
	27	76	39			13	37	13	
	28	81	40			14	44	15	
	29	65	43			15	65	25	

tures for the entire month of February at Manhattan, Kansas, ranged from 23° to 81° F. During the last ten days in February and the first three days in March the daily maximum had a range from 40° to 81° and the minimum from 25° to 43°. A sharp drop in temperature on March 4–5 lasted for eight days, as shown by the temperature records.

The minimum temperatures themselves would not have been injurious, if the evergreens had been in a dormant condition. The rather mild February in Kansas had stimulated activity in the tissues of vegetation. As a result, the sudden low temperatures and desiccating winds from March 5 to 13 were extremely injurious to plant life. Three to four weeks after the low temperatures, the injury became apparent on the evergreens. At first the foliage began to turn a tan or brown color. The affected areas began to dry. Even the woody tissue of some of the most susceptible varieties was killed. In the nurseries of Kansas the young trees were badly injured. The freezing temperatures burst the bark just above the ground line. In shrinking, the bark loosened and girdled the stems. On trees three feet tall and taller, the bark loosened only part way around the stem, so that there was sufficient cambium remaining to sustain life.

Among the varieties of Arborvitae it was observed that those with the " golden " or yellow foliage suffered the least. It was interesting to see, however, that the American Arborvitae, *Thuja occidentalis,* had practically no winter injury, while the variety of American Arborvitae, *Thuja occidentalis* var. *douglasii aurea,* was severely injured.

Among the Junipers only five varieties showed some injury. In the case of the Chinese Junipers most specimens escaped with only slight injury, although instances were observed where moderate to severe injury occurred. The Spiny Greeks, *Juniperus excelsa stricta,* were practically a total loss and show that the young trees were extremely sensitive. The Japanese Trailing Juniper, *Juniperus chinensis procumbens,* both variegated and green types, was slightly injured only in exposed locations. The foliage was killed in most instances, but new foliage has developed. The specimens of Japanese Red Pine, *Pinus densiflora,* which were observed were very severely injured. The remainder of the Junipers, both upright and prostrate types, and most of the pines, firs, and spruces escaped with practically no injury.

It is not the purpose of this paper to discuss winter injury to trees in general, but one may gain some idea of the severity of this freezing injury by the damage which resulted to the American elm. These trees were severely injured in many parts of the state. This seemed to be due to the loss of all vegetative buds on these twigs rather than the complete killing of the wood. A large portion of the last year's wood was completely killed, so that many trees have been severely weakened.

SUMMARY

A rather mild February in Kansas, 1932, stimulated activity in the tissues of vegetation. This warm spell was followed by a period of low temperatures

TABLE I. *A list of the Arborvitae and Junipers grown in Kansas and the freezing injury which resulted in the spring of 1932* *

Variety	Percentage of trees killed by freezing injury	Degree of injury to trees not resulting in death
Baker's Arborvitae, *Thuja* sp.	80 to 85	Very severe.
Berckman's Golden Arborvitae, *Thuja orientalis aurea nana*	25 to 30	Severe.
Chinese Arborvitae, *Thuja orientalis (Biota orientalis)*	20 to 25	Severe.
Var. Chinese Arborvitae, *Thuja orientalis* var. *compacta* (v. *sieboldii*)	50 to 75	Very severe.
Goldspire Arborvitae, *Thuja orientalis aurea conspicua*		Very severe.
Var. Chinese Arborvitae, *Thuja orientalis* var. *beverleyensis*		Very slight.
Var. Chinese Arborvitae, *Thuja orientalis* var. *bonita*		Very severe.
Var. Chinese Arborvitae, *Thuja orientalis* var. *meldensis*		Severe to very severe.
Nelson's Blue, *Thuja orientalis*		Severe.
Thuja orientalis elegantissima		Very slight.
American Arborvitae, *Thuja occidentalis*		Practically no injury.
Var. American Arborvitae, *Thuja occidentalis* var. *douglasii aurea*		Severe.
Rosedale Arborvitae hybrids, *Thuja* hybrids		Severe.
Bluegreen Arborvitae, *Thuja* sp.		Very severe.
Chinese Juniper, *Juniperus chinensis*		Slight to moderate.
Var. Chinese Juniper, *Juniperus chinensis* var. *albo-variegata*		Slight.
Japanese Trailing Juniper, *Juniperus chinensis procumbens*		Slight.
Common Juniper, *Juniperus communis*		Slight to moderate.
Spiny Greek, *Juniperus excelsa stricta*		Very severe.
Japanese Red Pine, *Pinus densiflora*		Very severe, but trees made a remarkable June recovery.
Jack Pine, *Pinus banksiana*		Slight.
Western Yellow Pine, *Pinus ponderosa*		Slight.
California type		Slight.
Colorado type		No injury.

* The nomenclature is primarily that used by nurserymen.

and desiccating winds from March 5 to 13. The reaction of species and varieties of Arborvitae and Junipers to injury was very striking. A list of 24 evergreens is given with a note on winter injury. Among the varieties of Arborvitae it was observed that those with " golden " or yellow foliage suffered somewhat less. Only five varieties of Junipers among those observed showed some winter injury. Most of the pines, firs, and spruces escaped with little or no injury.

The writer is indebted to Mr. Chas. A. Scott, McPherson, Kans., and Mr. Robt. Scott, Manhattan, Kans., for observations in their nurseries. Prof. L. R. Quinlan of the Department of Horticulture, Kansas State College, also gave the writer the results of his observations.

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RELATIONS OF TIME AND MAINTAINED TEMPERATURE TO GERMINATION PERCENTAGE FOR A LOT OF RICE SEED [1]

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INTRODUCTION

In Haasis's (1928) paper on the germinative energy of lots of seed of pitch pine and of other coniferous trees it is stated that a lot of rice seed was included in the experimentation there described, which was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University in 1927 and 1928. The present paper presents the main results of the experiments with rice there referred to, together with some discussion of relations between time, temperature, and germination percentage. In the preparation of this article we have had critical and bibliographic help from Dr. Thomas J. Edwards, of the School of Hygiene and Public Health, of the Johns Hopkins University.

The standard cultures of rice were essentially like the standard cultures of pitch pine seed described by Haasis, and those employed in Edwards's (1933) study of soybean seed were similar in many respects. Each culture regularly contained 50 seeds, taken as a random sample of the lot. After an hour of preliminary soaking, at about 20° C., the seeds were uniformly distributed on agar plates in covered Petri dishes. To the water in which the seeds were to be soaked was added 0.25 g. of " Semesan " powder for each 100 ml. According to the statement of the manufacturers, this material contained 35 per cent, by weight, of hydroxy-mercuri-chloro-phenol. For the agar plates, the granulated preparation called " Bacto-Agar " was used, 10 g. for each liter of water, and each culture dish contained 20 ml. of the mixture.

A series of tests at temperatures between 8° and 24° included seed samples soaked in distilled water as well as those soaked in antiseptic solution,. but there was no indication of any significant difference between the results from the two soaking treatments. We may regard the antiseptic treatment as without considerable effect on the results of this study, but it was a regular feature of the experimental background. Tests with seed samples that had not received any preliminary soaking at all gave results that were essentially like those secured with soaked seeds, excepting that the time period needed to give any specified germination percentage with a given maintained temperature was somewhat longer when unsoaked seeds were used, as might be expected. When hulls were removed from the seeds before they were distributed on the plates, without preliminary soaking, the results were like those secured when unsoaked seeds were used with hulls still present.

The air over each agar plate was practically saturated with water vapor throughout the incubation period. Light was excluded from the incubation chambers (Livingston and Fawcett, 1920) excepting for the short periods of observation. The chamber temperatures were approximately maintained, generally with less than a centigrade degree of fluctuation above and below the mean, but greater changes occurred at the times of observation, when the cultures came to room temperature for short periods. Seven different incubation chambers were used in each experiment. All the standard experiments were performed between October 14 and December 24, 1927. Since there is no evidence that the date of any series or the particular chamber used in any test (when different chambers were employed for the same temperature) exerted any influence on the results, all of the tests are considered as though they had been carried out simultaneously.

Aeration of the cultures was not taken as an experimental variable. This general condition embraces not only oxygen supply to the seeds and to the solution but also the escape of carbon dioxide and any other volatile substances that might perhaps be produced during incubation. While degree of aeration should of course be included among the experimental variables in a study such as this, if a fairly complete survey were proposed, it seems likely that aeration differences between these cultures were not influential. At any rate, whatever gas exchange took place was limited in its variation and fluctuation for any temperature by the technique of employing covered Petri dishes and 50-seed agar plates, as described. When 100 seeds, instead of 50, were used in each culture (thus presumably doubling the aeration requirement for germination with each combination of temperature and time), the results were like those from the regular series, temperature for temperature and period length for period length. Also, germination percentages were essentially the same whether the seeds rested on the surface of the agar plate or were partially or wholly buried in the gel. Apparently the degree of aeration was not low enough in the standard cultures, even with the higher temperatures tested, to be effective as a limiting condition. Higher degrees of aeration than were provided by the standard technique were not tested at all.

In this general connection it may be recalled that rice seed, like seed of some other swamp or water plants, has been observed to germinate well with almost no oxygen supply. In the Hopkins laboratory rice seeds have produced shoots as much as 3 cm. long when submerged in boiled water under oxygen-free nitrogen in hermetically sealed glass tubes, and seeds of water cress (*Roripa nasturtium*) have given similar results. Hutchins (1926; also Livingston, 1928) demonstrated quantitatively the very low oxygen requirement for germination of his rice seed planted very deep in wet, firmly packed soil. For a temperature about 22° the oxygen requirement for protrusion of the coleoptile was found to be lower than 0.00005 mg. per hour per square centimeter, while the corresponding oxygen requirement for his wheat seed was found to be about 0.00030 mg. On germination of rice seed with very

low oxygen pressure, the following authors are mentioned by Lehmann and Aichele (1931, p. 467–469): Demoussy (1907), Nagai (1916), Takahashi (1905), Yokoi (1898). Also see Sasaki (1927). A few other plants whose seed has been reported to germinate with very low oxygen pressure are: *Alisma plantago* (Crocker and Davis, 1914), *Cynodon dactylon* (Morinaga, 1926b), *Euryale ferox* (Okada, 1930), *Nelumbo nucifera* (Ohga, 1926), *Poa compressa* (Morinaga, 1926a), *Roripa nasturtium* (Morinaga, 1926a), *Trapa natans* (Terasawa, 1927), *Trifolium repens* (Morinaga, 1926a), *Typha latifolia* (Morinaga, 1926b).

The maintained temperatures employed in the regular cultures of the study here reported were between 8° and 55°, there being 26 of them, which are shown in table 1.

The lot of rice seed used was kindly given by Dr. E. B. Copeland, of Chico, California. It was of the "French" variety, grown in 1926 and received at Baltimore in April, 1927.

Observations and records were made at suitable intervals, showing how many of the 50 seeds of each culture had germinated in the last interval. A seed was considered to have germinated when the white tip of the coleoptile had emerged from the ruptured seedcoat, and all germinated seeds were removed from each culture at each observation. No seedlings advanced very far beyond this stage of germination, but in a number of instances the coleoptile projected several millimeters beyond the seedcoat, germination having apparently occurred early in the interval just ended. It is thus seen that each record showed how many of the 50 seeds of the culture in question had germinated in the corresponding interval *or had slightly passed the end-point of germination* as defined for this study. With more frequent observation these excesses of growth might of course have been avoided, but that would necessarily have introduced more frequent disturbances of temperature and the conditions of aeration and light, which might have introduced complications more troublesome than were occasioned by a few instances of growth excess. Neither the temporary temperature-light-aeration disturbances introduced at the times of observation nor the small amounts of post-germination growth here mentioned are considered as significant in this paper.

The increments of germination percentage were cumulatively summed so as to give the total number of seeds that had germinated in each incubation period. These totals were expressed as percentages of 50, the original number of seeds in each culture. Finally, all corresponding percentage values were averaged to give a mean germination percentage for each tested combination of maintained temperature and length of incubation period.

Thirteen different lengths of incubation period were employed, but only 225 of the different combinations of 26 temperatures with 13 time periods were actually tested. The mean percentage values for these are set forth in table 1, in which the temperatures are given in the first column and the lengths of period are read in the first line. The number of cultures upon which each

TABLE 1. *Mean germination percentages for different combinations of maintained temperature and length of incubation period, for standard cultures. (The subscript numeral indicates how many tests are represented by the mean.)*

Maintained temperature	Length of incubation period												
	9 hr.	12 hr.	18 hr.	1 da.	33 hr.	2 da.	3 da.	4 da.	5 da.	6 da.	7 da.	10 da.	14 da.
Deg. C.													
8	—	—	—	0_2	—	0_2	0_2	0_2	0_2	0_2	0_2	—	—
12	—	—	—	0_2	—	0_2	0_2	0_2	0_1	14_1	18_1	—	—
15	—	—	—	0_8	—	0_2	2_1	11_2	50_1	90_1	98_1	96_1	96_1
16	0_6	0_2	0_2	0_8	0_1	1_1	7_1	44_3	81_1	93_3	95_1	96_1	—
18	0_6	—	—	0_4	—	5_1	29_2	77_2	96_1	99_2	99_2	99_2	98_1
20	0_6	0_2	0_2	0_2	6_1	9_1	64_4	94_1	95_5	96_6	98_4	—	—
21	—	—	—	1_2	—	32_2	84_3	97_2	97_2	100_1	100_2	—	—
22	—	—	—	0_6	—	24_1	95_3	97_3	98_3	99_3	99_3	—	—
24	—	—	—	6_6	—	66_4	92_2	96_1	95_3	95_5	96_3	96_1	95_1
25	0_4	0_4	0_4	2_4	8_1	64_4	95_1	97_2	97_3	96_1	—	96_8	98_1
26	—	—	—	2_4	—	78_6	98_6	99_8	99_3	98_3	99_3	98_1	—
27	0_2	0_2	2_2	15_3	60_1	96_5	98_5	99_8	100_1	100_4	100_2	—	98_1
28	0_2	0_2	8_1	3_1	—	95_3	97_7	100_2	100_3	100_1	—	—	—
29	—	—	—	11_4	—	91_4	100_1	99_2	99_3	99_2	100_1	100_1	100_1
31	—	—	—	5_5	—	90_1	96_5	98_2	99_3	99_3	99_3	98_1	14_1
33	—	2_2	13_3	31_1	94_1	95_5	—	—	98_2	98_2	—	—	—
34	2_1	2_1	14_1	32_2	—	96_1	96_1	97_2	97_2	98_2	98_1	98_1	98_1
35	—	—	—	24_2	—	95_3	96_1	98_2	98_2	98_2	—	—	—
36	2_1	3_3	15_5	32_1	86_1	92_4	97_2	99_3	99_2	100_1	100_2	100_1	100_1
37	0_2	2_1	16_1	55_5	—	96_5	96_1	16_6	18_6	20_6	20_2	14_1	14_1
41	0_2	0_2	3_2	3_3	2_1	17_7	14_6	8	8	6	6	6_1	—
42	—	—	0_2	2_4	—	5_1	8	0_2	0_2	0_1	—	—	—
45	—	—	—	0_2	—	0_2	0_2	0_2	0_2	0_1	0_1	0_1	—
48	—	—	—	0_2	—	0_2	0_2	0_2	0_2	0_1	—	—	—
51	—	—	—	0_2	—	0_2	0_2	0_2	0_2	0_2	0_1	0_1	—
55	—	—	—	0_2	—	0_2	0_2	0_2	0_2	0_2	0_1	0_1	—

mean is based is indicated by a subscript numeral. Of course the value o indicates that no seeds showed protruding coleoptiles for the corresponding temperature-time combination. Dashes in the table indicate temperature-time combinations that were not tested.

DISCUSSION

General considerations

This study deals with the physiology of the organism as a whole rather than with details of the germination process; it belongs in the general field of ecological physiology or physiological ecology. Numerous presumably like samples of the given lot of seed are considered, but the individuals of a sample are not supposed to be alike. The general consistency of the results presented in table 1 supports the supposition that the several 50-seed samples were very nearly alike and that they generally represented fairly well the main lot of seed from which they were taken at random. Tests with 100-seed samples gave results essentially like those made with 50 seeds on each plate. Therefore the percentage means shown in table 1 are taken to be representative of the entire lot of seed.

The behavior of any organism, or the performance of any group or lot or population of organisms in any time period, is dependent, as is now generally recognized, partly upon the combined or integrated influence exerted by all the effective environmental conditions acting together as an external conditional complex and partly upon the internal characteristics of the organism or group. Other lots of seed with these same treatments might be expected to give results more or less different from the results here presented. It is clear from table 1 that this lot gave different results with many different temperature-time treatments, and other sorts of culture technique might be expected to give germination percentages for this lot more or less unlike those obtained by the culture method here employed. The two experimental variables of this study, temperature and time, are rather definitely specified in the scheme of table 1 and the rest of the environmental conditions are not specified, but their nature and intensities are implied in a description of the experimental technique employed, which really refers to a fairly definite set of environmental influences. These non-temperature conditions were not generally very well maintained, and their fluctuations or alterations are also implied when temperature, time, and the culture technique employed are all taken into account.

It may be emphasized that the test temperatures were maintained nearly constant throughout the incubation period, with only temporary fluctuations at the times of observation; the possible influence of changing temperature is not dealt with in this study, although the general importance of temperature change is readily recognized, especially when the attempt is made to apply experimental data to the study of natural environments. Some simple forms of temperature fluctuation or alternation have been employed in the study of the

environmental relations of seed germination, but that aspect was not included in the plan of this study; the whole problem of environmental fluctuation constitutes a subject by itself, much more complex than the problem of influences of maintained conditions. While maintained temperature has but a single dimension, which may be represented on a linear scale, temperature change could not be comprehensively surveyed without including in an experiment plan at least three kinds of variables—extent of change, time rate of change, and direction of change.

Special mention may be made of the possible influence of temperature and time on the background complex of external influences. Of course the agar plates may have altered in various ways with the progress of the incubation period, as by the accumulation of material derived from the seeds, etc., but the chemical environment was not taken as an experimental variable. The use of agar plates in loosely covered Petri dishes may have resulted in the introduction of influences related to aeration. As to water supply, the standard agar plates showed no notable drying out at the end of the longest incubation periods with the highest temperatures employed, and it seems safe to suppose that water supply was more than adequate at all times and in all tests.

In the planning of these tests and in the discussion of the results obtained the time factor is treated definitely as an experimental variable, the duration of incubation. Whether time is an external or an internal condition need not be decided, but it is surely an influential condition in every experiment dealing with the conditional control of changes or processes. Its importance in studies of this kind has been given special attention by many writers and is implied by many others.

A review of the existing literature on germination percentages of lots of seed and on the manner in which these percentage values appear to be influenced by the environmental conditions of the germination tests and by the length of the period of incubation has recently been presented by Edwards (1932). The present paper contains but few citations.

In routine seed testing for agricultural or silvicultural purposes it is necessary that procedure should be simple and that conventions should be followed; consequently only one or a very few kinds of test are apt to be applied to any lot of seed. For example, in the Rules for Seed Testing of the Association of Official Seed Analysts of North America (1927, a pamphlet published anonymously and without an editor's name) the instructions for testing rice seed include the following specifications: The seeds are not to be preliminarily soaked. They are to be incubated between sheets of wet, blue blotting paper for a period of 6 days (with an observation made at the end of the first 3 days as well as at the end of the period). The temperature is to alternate between 20° (18-hr. interval) and 30° (6-hr. interval).

The employment of such a single set of environmental conditions for testing germination capacity enables the experimenter to compare different lots of seed without undue expenditure of time and effort, but it needs to be re-

membered that the results of such a comparison are to be referred to the specific environmental complex used in the tests from which they have been derived. A more complete picture of the various ways in which several lots of seed may differ is had when every lot is tested by means of a number of standard procedures, or with a number of environmental complexes, each lot being subjected to the same series of treatments. An ideal study of this kind, in the realm of physiological ecology, should involve many different environmental complexes, so selected as to cover adequately the whole range of possibilities, but then the number of experimental variables considered would be great and the number of individual tests would naturally be greater still. Although this sort of ideal is not yet approachable, even for such a simple process as seed germination, yet it may be kept in mind as a goal toward which many lines of biological research are steadily advancing. In germination studies like the one here reported only relatively few of the environmental possibilities are quantitatively considered and the background complex has to represent the numerous experimental variables that are not brought into focus.

Special considerations

The data of table 1 may be studied with reference to the manner in which the seedling-producing capacity of this lot of rice seed varied according to the influences, or sets of influences, exerted by a large number of different complexes of test conditions, each set of conditions comprising the external background complex together with one of the tested combinations of temperature and time. All mean percentage values of 94 or above are shown in boldface type in the table and their differences may be regarded as insignificant; i.e., all values above 93 and below 100 may be considered as so nearly 100 as to represent complete germination within the precision limits of such a limited study as this. It is seen that these very high values were generally obtained with periods of incubation that were shorter as the maintained temperature was higher, up to 27°; that temperatures from 27° to 37°, inclusive, gave these very high values for an incubation period of apparently about 2.5 da.; that all values for 41° and 42° are very low, being about alike for all period lengths from 2 da. to 14 da.; and that no germination is shown for 45° or above.

Optimal temperature ranges are shown as follows: for 9 hr., 34°–37°; for 12 hr., 18 hr., and 24 hr., 33°–37°; for 33 hr., 34°–37°; for 2 da., 27°–37°; for 3 da., 24°–37°; for 4 da., 22°–37°; for 5 da. and 6 da., 18°–37°; for 7 da., 15°–37°. (Tests with 12° and 8° were not continued after the seventh day.) For the shortest periods these ranges are narrow and they are broader as the period was longer, but the upper limit of the range was close to 37° for every period length. It is notable that no mean percentage value below 95 occurs in any line of the table beyond the first occurrence in that line of a value of 94 or above.

There is no clear evidence indicating a double temperature optimum for

any period length (see Haasis, 1928; Haasis and Thrupp, 1931), but the logical possibility of the occurrence of two separate optimal temperature ranges is not entirely precluded by these data; maybe such an occurrence might have been discovered if additional period lengths between 1 da. and 2 da. had been tested with narrow temperature intervals, for a double optimum is perhaps vaguely suggested for the 2-da. period, for which the percentages for 29° and 31° are at least noticeably lower than those for 27°, 28°, 33°, 34°, 35° and 37°.

The minimal temperature for any germination in this lot of seed was lower as the period of incubation was longer. For the 18-hr. period it was about 28°; for the 1-da. period, about 21°; for the 2-da. period, about 16°; for the 3-da. period, about 15°; for the 7-da. period, about 8°. Akemine (1914), whose thorough study on rice germination should be read in connection with the present article, reported germination of his rice seed in water at 13° but not at 10°, and Ocfemia (1924) obtained no germination of his rice seed in soil at 12°.

The maximal temperature for any germination in the present study was about 37° for 9 hr. and for 12 hr. of incubation, about 41° for 18 hr., about 42° for 24 hr. and for 33 hr., and it appears to have been above 42° but below 45° for still longer periods. Akemine reported some germination of his rice seed at 40° but none at 45° or 50°; Ocfemia obtained some seedlings at 40° but reported no tests at any higher temperature; Jones's (1926) tests of two varieties of rice seed, incubated on moist paper, gave some germination of just one variety at 42° but no germination of either variety at 50°.

The general relations shown by table 1 are like those shown by the results obtained with rice seed in the early experiments of Haberlandt (1875), and in the recent ones of Akemine (1914), Jones (1926), and Ocfemia (1924).

<div align="center">* * * * *</div>

Throughout the preceding paragraphs this heterogeneous lot of seed has been considered with regard to its capacity to produce seedlings (with their coleoptiles just protruding), and reference has been made to this capacity as it was influenced by the several different temperature-time combinations that were employed in the tests. It is clear, however, that the performance of a lot of seeds, which is like a population, can be nothing more nor less than an integration of the several behaviors of its individuals, and the data of table 1 may next be studied with respect to the manner in which different portions of the seed population showed differences in performance.

Considering any individual seed in any test, it may be supposed that the complex process called germination began soon after the beginning of the preliminary soaking and continued more or less rapidly and perhaps with fluctuations, at rates determined by the internal and external conditional complexes that prevailed. In many instances the coleoptile of the embryo finally protruded, thus completing the process of germination (as arbitrarily defined for this study) and producing a germinated seed or a seedling. In

other instances this consummation was not attained before the close of the test, but it is not to be supposed that there was necessarily no germinational activity in these latter instances; the physiological process of germination may not have started at all, it may have gone forward at a rapid rate for a time and then become retarded, or it may have gone on slowly throughout the longest test period—too slowly to produce a seedling by the end of that time. It is to be emphasized that promptness in protruding the coleoptile gives indication concerning only the *mean* rate of progress in germination for the whole period from the beginning of soaking to the appearance of the coleoptile.

As has been pointed out by Haasis (1928), Tang (1931), and Edwards (1933), prolonged incubation of a sample of seed under a specified set of environmental conditions, with frequent observation and removal of all germinated seeds, results in a classification of the seeds of the sample into physiological classes, according to their promptness in attaining the end-point of germination under the standard test conditions. Of course this classification cannot be achieved till the classified seeds have germinated, but one may look backward and realize that the seeds with which the test started must have been of different capacities for promptness of germination under the specified set of external influences.

For example, refer to table 1 and consider a representative sample of soaked seeds placed on agar plates and incubated at a maintained temperature of 20°. At the end of a 2-day period 9 per cent of them are removed, having reached the end of germination in that period. These may be called the $20°_{2-da}$ class. The seedlings of this category may be considered as nearly alike because they are in about the same developmental phase and the seeds from which they came must have been nearly alike at the outset, with respect to their capacity to germinate under these test conditions; for all were treated alike and all have performed about the same amount of developmental work in nearly the same time. At the end of the third day of incubation 55 per cent more of the original sample of seeds are removed, to form the $20°_{3d da}$ class. At the end of the fourth day of incubation the seeds of the $20°_{4th-da}$ class are removed, comprising 30 per cent of the original sample. Six per cent of the seeds still remain on the plates and these may constitute a residual class, to which still further experimental analysis might perhaps be applied. The sprouted seeds of each class are alike within well-defined limits of precision, with respect to the promptness with which they were produced under the specified conditions, and the several classes are just as definitely different among themselves. By starting new cultures at 1-day intervals, etc., several different classes of just germinated seeds might be made available at the same time, and these might be used for simultaneous tests on more advanced phases of development, such as solution-culture experiments, etc. In a similar manner any other effective maintained temperature might be employed to establish a more or less different series of classes. By the use of shorter time intervals the number of classes for any test temperature might of course be increased

and the individual seedlings of any class would then be still more nearly alike according to the criterion here considered.

All physiological classes that are based on a single temperature with different lengths of incubation period are mutually exclusive; they do not overlap. For example, no seedlings belonging in either the $20°_{2\text{-da.}}$ class or the $20°_{4\text{th. da.}}$ class are to be found in the group selected at the end of the third day of incubation, etc. But it should be noted that classes based on different temperatures of incubation are not to be regarded as mutually exclusive, for there is no reason for supposing that these classes do not generally overlap to at least some extent. Thus, the $37°_{18\text{-hr.}}$ class might contain seedlings that would have appeared, for instance, in the $20°_{2\text{-da.}}$ or the $20°_{3d\text{-da.}}$ class, had they been subjected to a test temperature of $20°$ instead of $37°$.

One of the greatest fundamental needs of present physiological science is for practicable procedures by which groups of like organisms, such as seedlings, may be selected for systematic experimentation. Many experimenters have taken steps toward fulfilling this need, as by employing pure-line seed grown in a specified locality, by sorting the seeds of a lot with respect to size and other readily apparent characteristics, by sorting them according to their individual weights, and by selecting apparently like plantlets after a considerable size has been attained. The classification test just outlined may furnish a valuable addition to our present repertory of methods by which physiological homogeneity in a group of plantlets may be approached. If a single suitable maintained temperature is employed the suggested method should be very easy of application. Lots of seedlings may be sorted in this way before much growth has occurred, and one or more of the resulting classes may be used, the rest being discarded or used in separate experiments. For many kinds of plants the barely sprouted seeds are readily handled (as with bone-tipped or glass-tipped forceps) without great danger of disturbing their internal state or physiological tone. This method should be specially applicable to seeds of the small-grained cereals like rice and wheat; it has been shown to be applicable to seeds of pitch pine and several other coniferous trees (Haasis, 1928) and to seed of soy-bean (Edwards, 1933).

This way of classifying a lot of seed may perhaps be useful in genetics and plant breeding as well as in the preparation of seedlings for physiological experimentation, for it is easily conceivable that the different physiological classes of seedlings thus secured might be genetically distinct in some respects. For example, the mature plants grown from sprouted seed of the $37°_{18\text{-hr.}}$ class might differ considerably from plants grown from seeds of the $37°_{2d\text{-da.}}$ class. The possibilities are very numerous and interesting, and perhaps important. Edwards (1933) has given some examples of the use of this kind of classification as reported in the literature.

It is seen at once that the criteria for this sort of classification are simply the increments of germination percentage. The 24-hr. increments from the first to the seventh day for each tested temperature, obtained from the values of table 1 by subtraction, are all shown in table 2. Apparent decrements of

1 or 2 (for which the smallness of the number of values averaged is responsible) are entered as 0, to avoid confusion. The maximal increment for each temperature is shown in boldface type and the maximal increment for each day period is marked by an asterisk.

For temperatures from 12° to 24°, the maximal increment for any temperature (boldface type in the table) is generally shown to have occurred earlier as the temperature was higher, and the same is indicated for temperatures from 25° to 37°, although the one-day period is obviously too long to show this feature directly for the temperatures of the latter temperature range. With temperatures between 25° and 37° the rate of seedling production was so rapid in every instance that high germination percentages were recorded at the end of the second day of incubation.

Within the first five days of incubation, the maximal increment for any day (marked with an asterisk in the table) was obtained with progressively lower temperature. For the first day it was given by 37°; for the second, by 28°; for the third, by 22°; for the fourth, by 18°; for the fifth, by 15°. The greatest daily increment recorded (92 per cent) is for 28° and the second day. The greatest increment for the first day is only 55 per cent (37°) and for the third day it is of similar magnitude, 60 per cent (22°). For the fourth day it is still lower, 48 per cent (18°). For the fifth, sixth, and seventh days it is 39 per cent, 40 per cent, and 8 per cent, respectively, all obtained with temperature of 15°.

As has been indicated above, the increment values of table 2 may be taken to represent the various proportions of the individuals in this lot of rice seed

TABLE 2. *Mean daily increments of germination percentage*

Tempera-ture	Percentage increments						
	1st da.	2d da.	3d da.	4th da.	5th da.	6th da.	7th da.
Deg. C.							
12	0	0	0	0	0	**14**	4*
15	0	0	2	9	39*	**40***	8*
16	0	1	6	**37**	37	12	2
18	0	5	24	**48***	19	3	0
20	0	9	**55**	30	1	1	2
21	1	**31**	—	—	—	—	—
22	0	24	**60***	13	0	3	0
24	6	**60**	29	2	1	1	0
25	2	**62**	28	4	0	0	1
26	2	**76**	17	2	0	0	—
27	15	**81**	2	1	0	0	0
28	3	**92***	3	1	1	0	0
29	11	**80**	6	3	0	0	—
31	5	**85**	10	0	0	0	1
33	31	**64**	1	2	0	0	1
34	32	**63**	—	—	—	—	—
35	24	**72**	0	1	0	1	0
36	32	**60**	5	1	0	0	—
37	**55***	41	0	3	0	1	0
41	3	**14**	0	2	2	2	0
42	2	**3**	3	0	0	0	0

that belonged in several physiological classes, according to their capacity for more or less prompt germination at the several tested temperatures and under the background conditions of these tests. For a useful sorting to secure several non-overlapping classes an observational interval shorter than 24 hr. would be required if a temperature above 24° were employed. With any specified temperature between 15° and 37° any desired number of physiological classes might presumably be established by a suitable selection of observation intervals.

It is interesting to note that about 8 per cent of the seeds of this lot germinated at 42°, while about 20 per cent germinated at 41°. The remaining individuals were not necessarily all killed by these supra-optimal temperatures, as will be shown farther on. It may seem probable that the 20 per cent composing the 41° class embraced the 8 per cent composing the 42° class, but that is of course conjectural.

The germination classes here considered may have been related to differences in genetic composition of the embryos, as has been suggested above, or they may have been related to environmental conditions that were effective in the period of seed development—conditions, for example, of nutrition or of water supply of the ovule or of the parent plant. The classification might of course be related to both of these categories of influences together. The question thus raised seems to be worthy of special study, for a rice variety or for some other plant form or forms.

*   *   *   *   *

Another way to study the data of table 1 is to enquire, for each effective temperature, what was the shortest incubation period giving any specified percentage value. As illustrations we may choose the percentage values 10, 50, and 90. For a maintained temperature of 12° it required about 6 days of incubation to give 10 per cent; for 27°, about 1 day; for 42°, about 3 days. To give a percentage of 50 it required about 5 days of incubation at 15°, about 2 days at 24°, or about 1 day at 37°. To give 90 per cent about 6 days of incubation were needed at 15°, about 3 days at 25°, or about 2 days at 31° or at 36°, etc. These period lengths are shown by the graphs of figure 1, which were constructed from the data of table 1 by interpolation with reference to both time and temperature. They enable one to ascertain approximately the length of incubation period needed to obtain a specified germination percentage with each temperature, also the approximate temperature needed to give a specified percentage with each length of incubation period. The lowermost graph shows the times required for germination by the most active seeds of the entire lot and the uppermost graph shows the times required by the least active seeds.

It should be noted that each fraction or percentage group of the seed population properly refers only to the specified temperatures (and to the standard background conditions of these experiments); for example, the 10-per-cent group for a given temperature may or may not comprise the same

individuals as the 10-per-cent group for another temperature. Also the 50-per-cent group for any specified temperature includes all of the 10-per-cent group for that temperature and the 90-per-cent group includes the corresponding 50-per-cent group. Furthermore, since the individuals of any percentage group for any specified temperature did not all require exactly the same length of incubation period, it is clear that any period length properly represents just the *least active* seeds of the group.
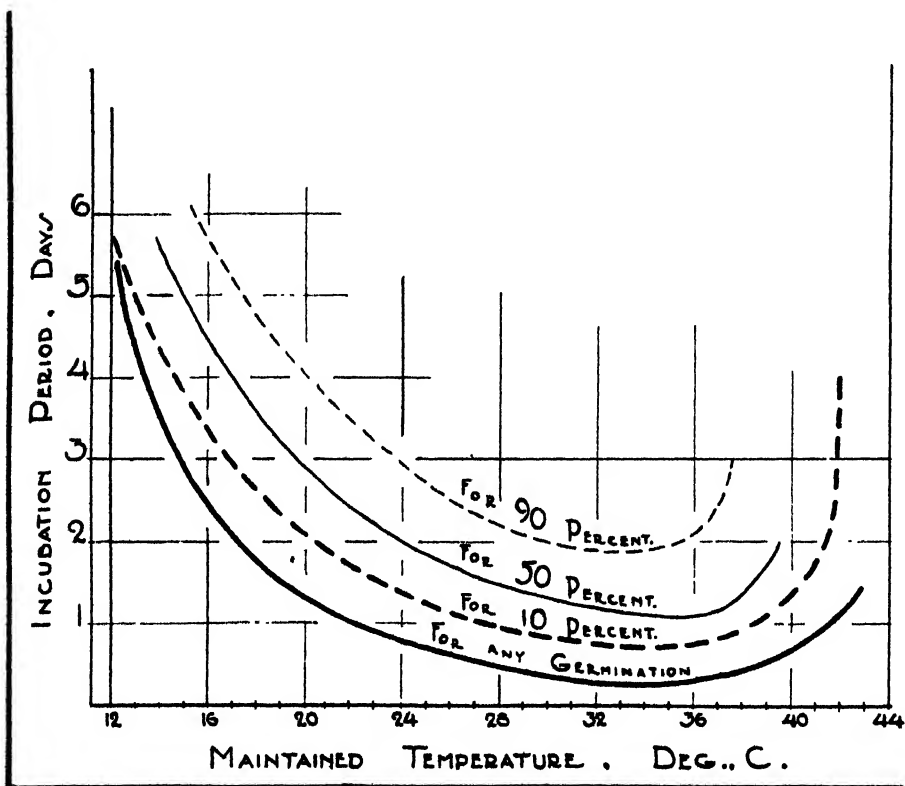
Fig. 1. Smoothed graphs showing for each maintained temperature the average lengths of incubation period required for any germination and for germination percentages of 10, 50, and 90.

It is apparently significant that the average length of time elapsing between the appearance of the most active embryos and the appearance of the least active ones is shortest for a temperature of about 34°, which is the temperature at which all the seeds of the lot appear to have been able to germinate most promptly.

Akemine's data (1914) on the germination of his rice seed have been used in the preparation of the graphs of figure 2, which are arranged like those of figure 1. The two figures are seen to be remarkably alike in their essentials. In view of a statement of Copeland's (1924, p. 131), that the variety called
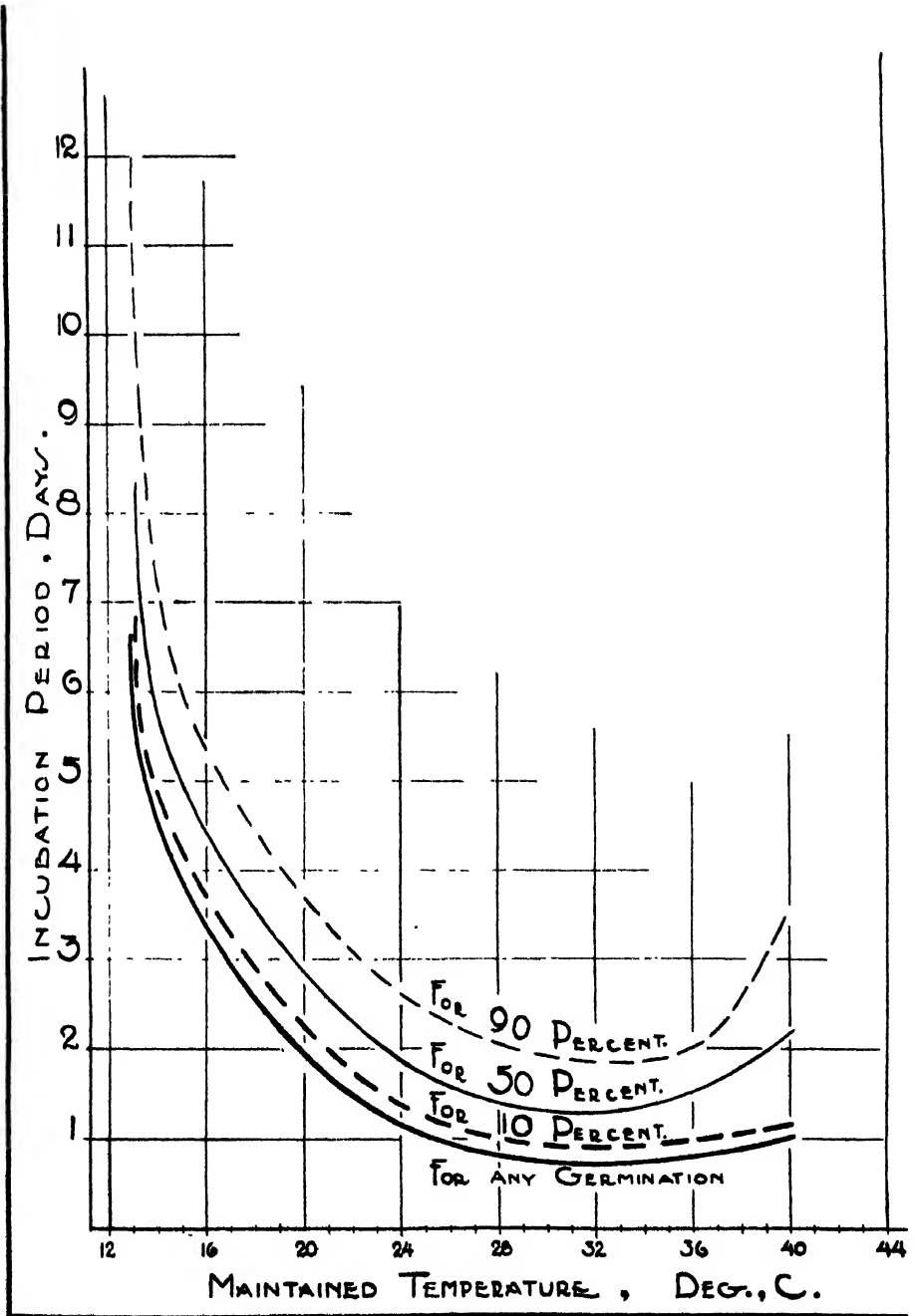
Fig. 2. Smoothed graphs similar to those of figure 1 but based on Akemine's (1914) data for his lot of rice seed.

" French " may contain " at least a dozen strains distinguishable in the field," it would not be surprising if there were far less accordance between these two sets of graphs.

<p style="text-align:center">*   *   *   *   *</p>

The average length of time required for germination may be estimated for each of the several effective temperatures and the resulting values may be regarded as a relatively simple system of physiological characteristics for this lot of seed.   Such averages may be secured by means of the following form of computation, which has been used by Haberlandt (1875), Akemine (1914), Edwards (1933), and others.   Each increment is multiplied by the length of the corresponding incubation, all the products thus obtained are added together, and the resulting sum is divided by the sum of all the increments. Using single-day increments, the following values were thus computed from the data of table 1, for the temperatures that gave practically complete germination within the period employed.

Maintained temperature	Ave. germination time	Maintained temperature	Ave. germination time	Maintained temperature	Ave. germination time
	Da.		Da.		Da.
15°	5.44	25°	2.38	33°	1.79
16°	4.68	26°	2.20	34°	1.66
18°	3.91	27°	1.89	35°	1.98
20°	3.41	28°	2.05	36°	1.75
22°	2.98	29°	2.01	37°	1.55
24°	2.95	31°	2.05		

Although a few small inconsistencies appear in this series of averages, they clearly indicate that the average incubation time was shorter as the temperature was higher, and the rate at which this average was shortened with progressively higher temperature is itself seen to decrease with higher temperature.   It should be noted that the 1-day observation intervals were too long for any high degree of precision in the mean increments on which these computed average times are based, especially for temperatures above about 27°. These average times appear to be in satisfactory agreement with some similar values given by Haberlandt (1875), Akemine (1914), and Ocfemia (1924), who experimented with seed of other rice varieties, employing culture methods some of which differed notably from those employed in the experiments reported in the present paper.

<p style="text-align:center">*   *   *   *   *</p>

Only about 20 per cent of the seeds of our lot protruded their coleoptiles when incubated at 41°, even with an incubation period of 14 days, and only about 8 per cent responded in 10 days with a temperature of 42°.   With a temperature of 45° no seedlings were produced in a period of 6 days.   The question therefore arises whether the seeds that failed to produce seedlings at these high temperatures were so altered internally as to be incapable of

completing the germination process if subsequently incubated with a lower and more generally favorable temperature. A few special tests were made bearing on this problem.

Cultures that had been incubated for 5 or 7 days at a temperature of 41°, with about 20 per cent of the seeds showing coleoptiles at the end of that period, were transferred to a temperature of 25° or 30°. Most of the remaining seeds germinated in a few additional days at the lower temperature. Incubation for a week at 41° does not, therefore, appear to have injured the seeds that failed to germinate in that period and at that temperature. But seeds first incubated for 5 days at a temperature of 45° or 51° showed no protrusion of coleoptiles at the end of that period and none after an additional incubation of 5 or 6 days at 30°. Seeds that had been incubated for 5 days with a temperature of 45° or above were apparently already dead by the end of that period, but it is of course conceivable that some special treatment (such, for instance, as high oxygen pressure, alternating temperature, etc.) might have caused·some of them to germinate even after the high-temperature treatment. Furthermore, with a temperature of 41°, the seeds that had not germinated in 14 days failed to germinate when subjected to additional incubation for 14 days at a temperature of 25°. It seems to have taken more than 5 days and less than 14 days of exposure to a temperature of 41° to prevent subsequent germination at 25° or 30°, but such subsequent germination was thoroughly prevented by prior incubation of only 5 days at a temperature of 45° or 51°. Those internal changes leading toward death which resulted from incubation at these high temperatures, in seeds that did not germinate under these conditions, seem to have progressed more rapidly at 45° than at 41°, as might be expected.

Cultures that had been incubated for a week at 12°, with only about 20 per cent of the seeds showing their coleoptiles by the end of that period, were then transferred to a temperature of 30° for an additional 3 or 4 days of incubation, which resulted in the protrusion of coleoptiles from most of the remaining individuals. When cultures were first incubated at 8° for a week (without any appearance of coleoptiles in that period) and were then transferred to a temperature of 30°, the seeds had nearly all germinated by the end of 3 or 4 days at the higher temperature. From such results as these it appears that low-temperature prevention of coleoptile protrusion for a week after soaking was not accompanied by any notable internal alteration in the seeds. Soaked seeds might be held apparently dormant for a week without any evidence of internal injury, if the protrusion of their coleoptiles in that time was prevented by these low temperatures. The physiological processes of these soaked seeds appear simply to have been held in abeyance, somewhat as in unsoaked seeds at ordinary temperatures. Edwards (1932) has called attention to several published records of retarded germination at about 0°, and Haasis and Thrupp (1931) have emphasized the retardative effect of the lower temperature in certain types of alternating-temperature treatments.

With the very high temperatures of this study, to go to the other end of the scale, some injurious processes, apparently leading to death in these soaked seeds, seem to have replaced the processes that would have led to the appearance of the coleoptiles if the temperature had been suitable for germination. Similar indications for Haasis's (1928) lot 1 of pitch pine seed have been recorded and discussed by him.

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Some special experiments were performed to compare Haasis's lot 1 of pitch pine seed with the lot of rice seed here dealt with, in regard to aeration requirement for the protrusion of the embryo from the seedcoat.   In the case of pitch pine the primary root appeared first through the ruptured coat, while the primary shoot appeared first in the case of rice.—As to this feature of the germination of rice seed, see Akemine's special study (1913).—The pitch pine seed showed a high oxygen requirement for germination, which agrees with the ideas prevalent among forestry nurserymen.   Deeply buried seeds of pitch pine (at the bottom of 50-ml. agar plates, in Petri dishes like those used for standard cultures) showed remarkable retardation in the production of seedlings, as compared with standard cultures, in which the seeds lay on the free surface of the regular agar plates.   After a 7-day incubation at 30° cultures of deeply buried pine seeds showed a germination percentage of only 2, while standard cultures of the same lot of seed showed a germination percentage of 92 with the same temperature-time treatment.   For the lot of rice seed here dealt with the percentage value for 30° and a 7-day period was above 90 for both standard cultures and cultures with deeply buried seeds. Aeration (presumably oxygen supply in good part) was apparently a limiting condition for the deeply buried cultures of pine seed and it was apparently not a limiting condition for the similar cultures of rice seed.

SUMMARY

A lot of " French " rice seed was studied.   Seeds were first soaked, then incubated on agar plates in Petri dishes, 50 seeds to the plate.   Twenty-six different maintained temperatures were tested, ranging from 8° to 55.° C. Germinated seeds were counted and removed from each plate at intervals for 14 days and the results are tabulated for 13 different lengths of incubation period.   Germination was considered as attained when the coleoptile just protruded from the seedcoat.   The primary numerical values considered are the mean germination percentages for the many different combinations of temperature and time.   From these are derived, for each tested temperature, mean increments of germination percentage for the several time intervals.

There is some discussion of environmental control of the performance of a lot or population of seeds, and variables other than temperature and time receive some general attention.   The data show that practically complete germination (94–100 per cent) was obtained within the 14-day period, for all tested temperatures excepting 8° and 12°, for low temperatures, and excepting

41°–55°, for high temperatures. For 9 hours of incubation the optimal temperature range was 34°–37° and the lower limit of this range was progressively lower as incubation was more prolonged, while its upper limit was 37° for every period length. For a period length of 7, 10, or 14 days the optimal range was 15°–37°. No clear evidence was shown for any double temperature optimum for any period of incubation. For an 18-hour period the minimal temperature for any germination was about 28° and this minimum was lower as the period was longer; for a 7-day period it was apparently somewhat above 8°. The maximal temperature for any germination was about 37° for a 9-hour incubation and it was higher with longer periods up to the 24-hour period, for which it was about 42°. For longer periods it was about the same, apparently above 42° but below 45°.

Special attention is given to the separation of the lot of seed into physiological classes according to promptness of germination at any specified favorable temperature. At 20°, for instance, 9 per cent of the seeds (the $20°_{2-da.}$ class) germinated within the first 2 days of incubation; at the end of the third day an additional 55 per cent (the $20°_{3d-da.}$ class) had germinated; and the $20°_{4th-da.}$ class embraced 30 per cent of the lot. ' By choosing a suitable temperature and suitable observation intervals, many different classifications may be had. If a single temperature is used and if new cultures are started at proper intervals, several mutually exclusive classes of just germinated seeds may be made available for simultaneous tests on more advanced phases of development, such as solution-culture experiments. It is pointed out that this sort of classification of a lot of seeds may furnish a useful addition to our present repertory of methods by which physiological homogeneity in a group of plantlets may be approached.

The classification just mentioned is based on time increments of germination percentage, and the mean daily increments for the several temperatures tested and for the first 7 days of incubation are studied in some detail. Within the first 5 days, the maximal mean increment for any day was obtained with progressively lower temperature; for the first day it was 55 per cent, obtained with 37°, for the fifth day it was 39 per cent, obtained with 15°. The greatest mean daily increment (92 per cent) was for 28° and the second day. At 41°, 20 per cent of the seeds germinated (in the first 6 days) and at 42°, 8 per cent germinated (in the first 3 days).

The shortest incubation period giving a specified germination percentage is especially studied and its variations are shown by graphs. For every percentage this shortest period is long for low and for high temperatures and shortest for about 34°. The mean length of time elapsing between the appearance of the most prompt and the appearance of the most tardy seedlings was also shortest for a temperature about 34°. All seeds germinated most promptly at about that temperature.

The average incubation time required for germination was computed for each of the several effective temperatures. It was shorter as the temperature was higher and its rate of shortening decreased with higher temperature.

A few special experiments dealt with the effect of prolonged incubation without germination, at low and at high temperatures. A comparison of the aeration requirements of this rice seed and of Haasis's pitch pine seed showed clearly that the aeration requirement for germination was very much higher for the pine seed. Buried at the bottom of agar plates for 7 days at 30°, the rice seed showed a germination of 90 per cent while the other seed showed only 2 per cent; but both kinds of seed showed a germination of about 90 per cent when incubated on the surface of the plates for 7 days at 30°. .

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SOME NEW OR OTHERWISE IMPORTANT COMPOSITAE OF THE HAWAIIAN ISLANDS

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Dubautia magnifolia sp. nov.—Verisimiliter frutex, ramis glaberrimis. Folia opposita oblongo-lanceolata, deorsum longe attenuata quasi petiolata inferne, basi subamplexicaulia subconnataque, glaberrima, infra pallida et subperspicue ∓ 9-nervia, supra coriacea vix subnitida et obscurissime nervia, marginibus eciliatis supra medium obscurissime subcrenulateque denticulata, apice longe acerrimeque acuminata, 1.5–2.6 dm. longa et 2.5–3.3 cm. lata. Capitula numerosa laxe in panicula pyramidata ∓ 2.5 dm. longa ramis longe molliterque patenti-pilosis disposita, tenuissime pedicellata pedicellis folioso-bracteatis bracteis oblongo-lanceolatis sparsim hispidis saepius 1–1.5 cm. longis. Involucrum cylindraceum vel cylindraceo-obconicum bracteis 5–8 glabris saepe purpurascentibus fere usque ad apicem valde connatis vel rarissime paucis subseparatis 5–6 mm. longis. Flores circ. 8, purpurascentes, corollae tubo gracili saepe arcuato quam limbo exserto triplo longiore. Achaenia sparsim hispida, corpore 3.5–4 mm. longa, pappi aristis tenuissimis sordidis sursum hispidis 3–4 mm. longis.

Type specimen: *J. F. Rock* 9012, Waialae Stream, Isl. Kauai, October, 1911 (Hb. Gray).

DUBAUTIA LAEVIGATA **parvifolia** var. nov.—Panicula ∓ 1 dm. longa, foliis principalibus 7–10 cm. longis et 8–14 mm. latis.

Type specimen: *Mann* and *Brigham* 538, Waimea, Isl. Kauai (Hb. Gray).

DUBAUTIA PLANTAGINEA **Chamissonis** var. nov.—A specie foliis basim versus plus minusve angustatis semiamplexicaulibus, paniculae ramis oppositis differt.

Type specimen: *A. Von Chamisso,* Isl. Oahu (Hb. Kew).

DUBAUTIA PLANTAGINEA **angustifolia** var. nov.—Folia angustiora, principalia circ. 1.2–1.9 dm. longa et 0.7–1.5 cm. lata; paniculae ramis oppositis.

Type specimen: *Dr. Wm. Hillebrand,* on high mountain to the right of the Pali of Nuuanu, Isl. Oahu, September, 1860 (Hb. Kew).

DUBAUTIA PLANTAGINEA **acridentata** var. nov.—Folia principalia usque ad 2.1 dm. longa et 4 cm. lata, basi angustata, utrinque pilis adpressis strigosa, marginibus apicem versus acriter dentata dentibus 1–2 mm. longis saepe patentibus. Paniculae rhachis valde setis albis sursum arcuatis in 8–12 lineis parallelis dispositis hispida, ramis oppositis.

Type specimen: *J. F. Rock* 6135, Maunahui Gulch, Isl. Molokai, Mar. 21, 1910 (Hb. Bishop Mus.).

[1] Published out of the order determined by the date of receipt of the manuscript.

DUBAUTIA PLANTAGINEA var. PLATYPHYLLA **occidentalis** f. nov.—Foliorum dentes parvi, nec spinulosi nec patentes.

Type specimen: *Mann* and *Brigham* 365, mountain of west Maui (Hb. Bishop Mus.).

✕ **Dubautia media** hybr. nov.—Inflorescentia *D. laxae* ipsi exacte similis. Folia (unico ramo viso) sub inflorescentiam paulo exsertam conferta petiolis imbricatis, oblongo-oblanceolata, inferne sensim ad basim quasi petiolata angustata, apice acuminata, margine eciliata sed obscure denticulata, faciebus pallida subcoriacea 7–15 nervia nervis parallelis subobscuris, habitu illis *D. plantagineae* var. *Chamissonis* similia. Ramus ipse infra folia glaber, ligneus, 4–6 mm. crassus, foliorum delapsorum cicatricibus confertis (intervallis superioribus plerumque 1–6 mm. inferioribus plerumque ∓ 8 mm. longis) eciliatis.

Type specimen: *H. N. Wells,* eastern end of Isl. Kauai (Hb. Field Mus.).

✕ **Dubautia mendax** hybr. nov.—Foliis inter *D. laevigatam* et *D. laxam,* iis supra glabris infra marginaliterque adpresso-hispidis, principalibus ∓ 1 dm. longis et ∓ 1.5 cm. latis. Inflorescentia paniculata plus minusve contracta, capitulis ut in *D. laxa,* disco demum circ. 6.5 mm. alto, bracteis hispidis, corollae tubo glanduloso. Probabiliter inter *D. laevigatam* et *D. laxam* hybrida.

Type specimen: *C. N. Forbes* 1042K, Waimea Drainage Basin, west side, Isl. Kauai, Jul. 3-Aug. 18, 1917 (Hb. Bishop Mus.).

DUBAUTIA WAIALEALAE **megaphylla** var. nov.—Folia faciebus glabra, apicem versus acriter serrulata, 3.5–5.3 cm. longa et 7–11 mm. lata.

Type specimen: *J. F. Rock* 5022, Mt. Waialeale, Isl. Kauai, September, 1909 (Hb. Gray).

DUBAUTIA LAXA **hispida** var. nov.—Folia oblanceolata, utrinque adpresso-hispida, inflorescentia exserta, involucri bracteis valde hispidis.

Type specimen: *Charles Gaudichaud* (*Voy. Bonite*) 218 *pro parte,* September-October, 1836 (Hb. Delessert).

DUBAUTIA LAXA var. OBOVATA **glabrescens** f. nov.—Folia supra et interdum etiam infra glabrescentia.

Type specimen: *U. S. S. Pacif. Explor. Exped. under Capt. Wilkes,* mts. behind Honolulu, Isl. Oahu, 1840 (Hb. N. Y. Bot. Gard.).

DUBAUTIA LAXA **Bryanii** var. nov.—Folia oblanceolata, glabra, 8–12 cm. longa. Capitula 5–8-flora, parva, involucri bracteis 5–8, tantum circ. 2–3 mm. longis. Achaenia corpore circ. 1.9–2.2 mm. longa, aristis interdum paulo longioribus.

Type specimen: *Forbes* and *Cook,* Koolauloa Mts., between Puualuu and Kaipapau, Isl. Oahu, May 3–8, 1909 (Hb. Field Mus.).

Named for Mr. Edwin H. Bryan, Jr., of the Bernice P. Bishop Museum, in Honolulu. Mr. Bryan generously supplied me with much herbarium material of this and other forms.

DUBAUTIA MICROCEPHALA **Forbesii** var. nov.—Capitula majora. Involucri bracteae 4–5.5 mm. longae nunc obovatae nunc lineari-oblongae. Corollae limbus plus minusve urceolatus. Achaenia angustiora, corpore 3–3.5 mm. longa.

Type specimen: *C. N. Forbes* 399a, Kaholuamanu, Isl. Kauai, September, 1909 (Hb. Bishop Mus.).

RAILLARDIA [2] MENZIESII **angustifolia** var. nov.—Folia opposita, linearia vel anguste oblongo-elliptica, 3–5.5 cm. longa et 5–12 mm. lata.

Type specimen: *J. F. Rock* 8546, alt. 6000 ft., Mt. Haleakala, Isl. Maui, September, 1910 (Hb. Gray).

Raillardia thyrsiflora sp. nov.—Forsitan frutex; ramis inferne ligneis supra herbaceis, plus minusve subpurpurascentibus, glabratis, infra confertissime supra laxe foliosis internodiis inferioribus vix 1–2 superioribus ∓ 1 cm. longis. Folia superiora suberecta alterna vel interdum opposita alia saepe subterna plerumque demissa, omnia sessilia, anguste vel late linearia, faciebus glabra et 3-nervia, sicca plus minusve brunneo-purpurea, marginibus ciliatis plus minusve revoluta et utroque minutissime 1–3-denticulata, principalia 3–4 cm. longa et 4–8 mm. lata. Capitula sublaxe vel subconferte in panicula erecta villosa foliosa usque ad 1.5 dm. longa disposita, interdum nonnulla ad anthesin cernua, tenuiter pedicellata pedicellis ultimis 4–9 mm. longis, ∓ 8-flora corollis subflavidis non exsertis, genitalibus inclusis 11–12 mm. longa. Involucrum cylindraceo-obconicum, sparsim hispidum, 7–8.5 mm. longum, bracteis 6–8, satis connatis itaque lobis acutis 1–2 mm. longis. Achaenia nigra, oblanceolata, glabra, inferne sensim angustata, corpore ∓ 3 mm. longa, pappi sordidi aristis plumosis ∓ 5 mm. longis.

Type specimen: *C. N. Forbes* 1203*M*, north slope of Mt. Haleakala, Isl. Maui, Aug. 23, 1919 (Hb. Bishop Mus.).

RAILLARDIA THYRSIFLORA **cernua** var. nov.—Rami elongatiores. Folia alterna, 5–6.5 cm. longa et circ. 5–6.5 mm. lata. Panicula laxior, ∓ 2 dm. longa, capitulis ad anthesin plerumque cernua.

Type specimen: *C. N. Forbes* 1233*M*, north slope of Mt. Haleakala, Isl. Maui, Aug. 23, 1919 (Hb. Bishop Mus.).

Raillardia ternifolia sp. nov.—Forsitan frutex; unico ramo viso herbaceo, subarcuato-recto, brunnescenti, plus minusve angulato, subsparsim nunc erecte adpresso- nunc adscendenti-hispido, inflorescentia adjecta circ. 4 dm. longo, internodiis inferioribus 1–1.5 cm. superioribus 3–5 cm. longis. Folia omnia perspicue terna, suberecta, sessilia et subamplexicaulia, oblongo-linearia vel anguste lanceolato-linearia vel oblanceolato-linearia, longitudinaliter 5-nervia nervis supra depressis, pallida, supra subcoriacea subnitidaque, utrinque glabra, acriter hispido-ciliata et utroque margine supra medium 1–6-denticulata dentulis subpatentibus, 4.5–6 cm. longa et 7–8 mm. lata. Capitula subracemose aggregata in panicula sublaxa foliosa erecta ∓ 1.9 dm. longa, cum genitalibus circ. 1.1 cm. longa, ∓ 8-flora. Involucrum cylindraceum vel cylindraceo-obconicum, viride vel vix subpurpureum, adpresso-erecte plus minusve setosum, 6–7 mm. longum, bracteis 5–8, valde connatis itaque lobis subacutis circ. 1 mm. longis. Flores ochroleuci, corollae glabratae limbo non exserto. Achaenia submatura linearia, glabra, corpore circ. 4 mm. longa, pappi vix subpurpurascentis aristis plumosis ∓ 5 mm. longis.

Type specimen: *C. N. Forbes* 1175*M*, north slope of Mt. Haleakala, Isl. Maui, Aug. 20, 1919 (Hb. Bishop Mus.).

RAILLARDIA MONTANA **longifolia** var. nov.—Rami brunneo-subpurpurascentes. Folia opposita, patentia, subrhomboide elliptico-lanceolata, aegre coriacea, non nitida, glabrata, 5- vel 7-nervia, 5–6.5 cm. longa. Capitula circ. 8–10-flora. Achaenia corpore 4.5–5.5 mm. longa.

Type specimen: *J. F. Rock* 8603, Kaupo Gap, Mt. Haleakala, Isl. Maui, Oct. 22, 1910 (Hb. Gray).

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[2] Asa Gray's altered spelling (Proc. Amer. Acad. **5**: 132. 1861) of Gaudichaud's *Railliardia* is here followed only tentatively.

RAILLARDIA MONTANA **robustior** var. nov.—A specie foliis coriaceis saepe ovato-ellipticis vel anguste cuneato-obovatis paulo majoribus differt.

Type specimen: *J. F. Rock* 8594, Puunianiau, Mt. Haleakala, Isl. Maui, Oct. 11, 1910 (Hb. Gray).

RAILLARDIA DEMISSIFOLIA **verticillata** var. nov.—Folia principalia saepius terna.

Type specimen: *C. N. Forbes* 1171*M*, north slope of Mt. Haleakala, Isl. Maui, Aug. 17, 1919 (Hb. Bishop Mus.).

RAILLARDIA CILIOLATA var. **laxiflora** comb. nov.; *R. laxiflora* DC.　Prodr. 6: 441.　1837.

RAILLARDIA MOLOKAIENSIS **oppositifolia** var. nov.—A specie foliis principalibus plerumque oppositis differt.

Type specimen: *U. Faurie* 936, Kamalo, Isl. Molokai (Hb. Deless., 2 sheets).

RAILLARDIA MOLOKAIENSIS **stipitata** var. nov.—A specie achaeniis longius hispidis inferne angustissime stipitatis differt.

Type specimen: *C. N. Forbes* 2603*M*, along upper trail, Waikamoi, Isl. Maui, June 25, 1920 (Hb. Bishop Mus.).

RAILLARDIA LINEARIS **opposita** var. nov.—Folia'opposita, utroque latere 1–3-denticulata.

Type specimen: *J. F. Rock* 6126, Maunahui Gulch, Isl. Molokai, Mar. 21, 1910 (Hb. Gray).

RAILLARDIA PLATYPHYLLA **leptophylla** var. nov.—Folia confertissima, anguste lanceolata, plerumque 6–7 cm. longa et 1–1.5 cm. lata.

Type specimen: *J. F. Rock* 8578, Puunianiau Crater, Mt. Haleakala, Isl. Maui, October, 1910 (Hb. Gray).

RAILLARDIA SCABRA **Munroi** var. nov.—Robustior, foliis principalibus 3-nerviis 5–7.5 cm. longis et 4–6 mm. latis.

Type specimen: *G. C. Munro* 747, alt. 4250 ft., edge of thick forest, Haleakala Ranch pipe line, east Maui, Nov. 23, 1927 (Hb. Bishop Mus.).

Raillardia lonchophylla sp. nov.—Habitu *Dubautiae plantagineae* Gaud. similis.　Rami sparsissime setosi, herbacei.　Folia conferta, alterna, oblongo-oblanceolata, apice acuminata, infra medium usque ad basim angustata quasi subpetiolata, pallida, longitudinaliter perspicueque 7–11-nervia, infra glabra supra secundum venas marginesque hispida, acriter denticulata dentulis patentibus sub 1 mm. longis, 8–12 cm. longa et $\mp$ 2 cm. lata.　Capitula in panicula villosa ramis adscendentibus patentibusve alternis disposita, gracillime pedicellata, circ. 6–8-flora, cum genitalibus circ. 1 cm. longa.　Involucrum obconico-campanulatum, subsparsim hispidum, bracteis 6–8 plus minusve connatis circ. 5.5–6 mm. longis.　Flores flavidi, corollae limbo non exserto.　Achaenia submatura hispida linearia deorsum usque ad basim sensim angustata, corpore vix 3 mm. longa, pappi aristis sordidis plumosis circ. 3 mm. longis.

Type specimen: *J. F. Rock* 8599, Laie, Kaupo Gap, Mt. Haleakala Crater, Isl. Maui, Oct. 22, 1910 (Hb. Bishop Mus.).

CHICAGO NORMAL COLLEGE,
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63

EFFECT OF MANGANESE DEFICIENCY ON THE GROWTH AND SUGAR CONTENT OF PLANTS [1]

LAWRENCE P. MILLER

Considerable work has been done tending to show that manganese is essential for the growth of green plants. The amount required is so small, however, that it has usually been necessary to use special precautions to eliminate the manganese present as impurity, in order to demonstrate the essential nature of this element. The effect of manganese deficiency on the metabolism of plants has not received much attention, presumably because of the extreme difficulty in growing a large number of manganese-deficient plants under controlled conditions.

As reported in a previous paper (Miller, 1928), in experiments with sand cultures, conditions of manganese deficiency were obtained without the use of the special technique usually considered necessary. The cultures employed were so low in manganese that plants growing from seed made no further growth after a few weeks unless manganese was supplied. Studies on the effect of manganese on the chemical constituents of various plants were begun but due to circumstances had to be discontinued. Results obtained up to the time the work was ended in 1929, showing that manganese has very important effects on the sugar content of plants, are reported in this paper.

METHODS

Sand cultures. The plants were grown in sand cultures in new red earthenware pots. The sand used was a pure quartz sand obtained from the Ottawa Silica Company. Nutrients were made up from the following stock solutions: $Ca(NO_3)_2 4H_2O$, 4 per cent; KNO_3, 2 per cent; $K_2HPO_4 3H_2O$, 2 per cent; $MgSO_4 7H_2O$, 2 per cent. The chemicals used were the purest grade obtainable from the Mallinckrodt Chemical Company. Each culture received 20 cc. of each of the above stock solutions in suitable dilution at weekly intervals, when the plants were grown in 12-inch pots. When 4-inch pots were used, only 5 cc. of each stock solution were added. Iron was supplied in the form of ferric citrate.

Chemical analyses. For sugar analyses the material was added to boiling 80 per cent alcohol to which $CaCO_3$ had been added. Subsequently the samples were thoroughly extracted with 80 per cent alcohol. The alcohol was

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evaporated off and the samples were cleared with lead acetate and deleaded with sodium oxalate. Reduction of Fehling's solution was carried out under the conditions recommended by Quisumbing and Thomas (1921) and the Cu_2O determined by titration with potassium permanganate after solution in sulphuric acid in the presence of ferric ammonium sulphate.

RESULTS

Effect of manganese on growth

As brought out in a previous paper, if plants were started from seed in the cultures described, growth practically stopped after a few weeks (Miller, 1928). The plants showed the symptoms typical of manganese deficiency (Schreiner and Dawson, 1927) and remained alive for a long time without any further development. If a small amount of manganese sulphate was added to the cultures, growth began at once, and in the case of the tomato such plants were grown to maturity with the production of fruit and seed.

Even if tomato plants were started in good greenhouse soil and grown in such soil until about ten inches high before being transferred to the sand cultures, manganese deficiency symptoms developed. The resultant growth in one such experiment is shown in table 1. Tomato seed was planted in a flat on January 3, 1928. The plants were transferred to good greenhouse soil in 4-inch pots on February 1. After growing in this soil for 19 days, they were transferred to the sand cultures in 12-inch pots. Three plants were grown in each pot. To half of the pots 30 mg. of $MnSO_4$ were added on February 19. To some of these 200 mg. more were added on March 22, while on April 2 some of the no-manganese cultures received 90 mg. of $MnSO_4$. Table 1 shows the average weight of leaves, petioles, and stems from plants of these experiments on March 18, March 28, and April 11. The figures represent averages of from 25 to 73 plants, except in the case of the sample from the 230 mg.-cultures taken on April 11, when only 15 plants were sampled.

Table 1 shows that on March 18, or after the plants had been in the sand cultures for about one month, the plants which had received no manganese had only about half the fresh weight of the plants from the pots to which 30 mg. of $MnSO_4$ had been added. The table also shows that the no-manganese plants made very little further growth in the period between March 28 and April 11. The no-manganese plants which received 90 mg. of $MnSO_4$ on April 2 responded very well, as can be seen when the weights of these plants on April 11 are compared with those to which no manganese had been added.

Further comparisons of the growth of various plants with and without manganese can be made from the data in the first part of table 2. These plants were started from seed in 4-inch pots. About 30 days from the time the seeds were planted, one-half of the cultures received 5 mg. of $MnSO_4$. The plants were sampled about 50 days later. The plants which had received

TABLE I. *Effect of manganese sulphate on the fresh weight of tomato plants grown in sand cultures*

Sampling date	Leaves, grams per plant				Petioles, grams per plant				Stems, grams per plant			
	No Mn	30 mg.	230 mg.*	90 mg.†	No Mn	30 mg.	230 mg.*	90 mg.†	No Mn	30 mg.	230 mg.*	90 mg.†
March 18........	6.68	12.67	—	—	2.75	5.79	—	—	3.21	6.82	—	—
March 28........	16.58	22.24	19.59	—	7.14	10.16	8.86	—	10.63	15.94	13.14	—
April 11........	17.08	35.71	36.67	26.10	8.12	22.07	20.40	11.97	12.12	35.50	30.67	18.84

* 200 mg. MnSO₄ added to the 30 mg. cultures on March 22.
† 90 mg. MnSO₄ added to the no-Mn cultures on April 2.

TABLE 2. *Effect of manganese on the sugar content and dry weight produced in various plants grown in sand cultures*

Plant	Dry weight per plant				Reducing sugars, % of dry weight				Sucrose, % of dry weight			
	Tops		Roots		Tops		Roots		Tops		Roots	
	With Mn	Without Mn	With Mn	Without Mn	With Mn	Without Mn	With Mn	Without Mn	With Mn	Without Mn	With Mn	Without Mn
Wheat............	0.44	0.07	0.52	0.14	1.63	*	1.08	*	8.32	1.60	3.32	*
Corn.............	0.99	0.39	0.59	0.29	2.48	1.27	2.82	1.12	4.84	2.39	7.16	2.88
Lettuce..........	0.44	0.07	0.21	0.02	4.42	*	0.32	*	9.54	4.96	6.46	*
Cabbage.........	0.32	0.17	0.20	0.07	—	—	—	—	—	—	—	—

* In these cases the sugar content was so low as to give no copper precipitate in the sample taken for analysis.

manganese had a dry weight from two to ten times that of the no-manganese controls. Of course if these plants had been sampled later, the differences would have been greater still, since the plants without manganese would have made little if any further growth while the ones which had received manganese would have grown to maturity.

Other plants which showed manganese deficiency under these conditions were barley, rye, and tobacco. No species tested failed to give these results.

In figure 1 are shown some tobacco plants grown from seed in sand cultures. The plant in the center received 5 mg. of MnSO₄ about one month

Fig. 1. Tobacco grown from seed in sand culture with and without the addition of MnSO₄. The plant in the center received 10 mg. of MnSO₄.

after planting and another 5 mg. a month later. The plants on either end were grown without the addition of manganese.

Introduction of manganese directly into the plants

In order to be sure that the favorable response resulting from the addition of manganese was due to the action of manganese within the plant and not to some reaction between the manganese added and some constituent of the sand culture, small amounts of manganese sulphate were added directly into the stems of some tomato plants. A pyrex test tube was drawn out to a fine point; this was carefully inserted into the stem, and the manganese sulphate was added in a dilution of 1 part to 2000 parts of water. It usually took a day or so for the solution to be absorbed by the plant. In this way, using pots containing two plants, it was possible to have a manganese-starved plant and a plant with manganese added growing in the same pot. The environmental conditions of the two plants were thus exactly the same except for the addition of the manganese to one of the plants.

The result of such a test is illustrated in figure 2. A pot containing two plants showing marked symptoms of manganese starvation was selected and photographed on April 8. This is shown in figure 2 A. The plant on the

right side of the pot received 2 mg. of $MnSO_4$ on April 17. A second photograph, shown in figure 2 *B*, was taken on May 6, and it can be seen that the plant has already responded to the addition of the manganese. Further addi-
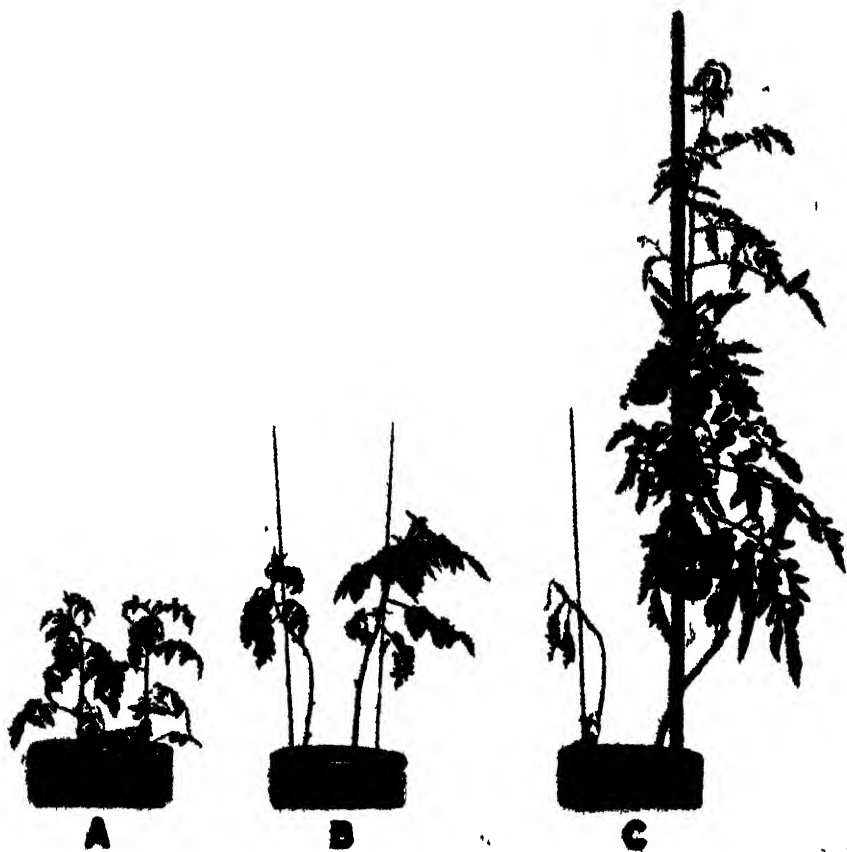
Fig. 2. Effect of the direct introduction of $MnSO_4$ into the stem of a tomato plant. A no-manganese culture photographed April 8 (*A*); May 6 (*B*); and June 23 (*C*). The plant on the right in each case received 2 mg. of $MnSO_4$ by direct introduction into the stem on April 17, May 6, and May 20.

tions of 2 mg. of $MnSO_4$ were made on May 6 and May 20. A photograph taken on June 23 (fig. 2 *C*) shows that this plant has made good growth and matured some fruit, while the plant which received no manganese has made no growth and is barely alive.

The addition of other elements besides manganese to the sand cultures failed to bring any response. Copper sulphate and boric acid were added in various amounts without any beneficial effects. Additional amounts of iron as ferric citrate also had no effect. Other manganese salts, such as the tar-

trate, oxalate, and citrate, brought about a response similar to that of the sulphate.

Effect of manganese on the sugar content

Analyses of plants which were grown with a small amount of manganese showed that these were often several hundred per cent higher in sugar content than corresponding plants to which no manganese sulphate had been supplied. The reducing sugar and sucrose contents of the tops and roots of wheat, corn, and lettuce grown with and without the addition of manganese sulphate are shown in table 2. In a few cases the sugar content of the no-manganese plants was so low as to give no precipitate of Cu_2O in the sample taken for analysis. In those cases in which figures are given, it is seen that the no-manganese plants contained only about one-half to one-fifth of the amount of sugar of the plants growing in cultures to which 5 mg. of $MnSO_4$ had been added. Analyses of tobacco leaves grown under similar conditions (not shown in table 2) gave the same results.

Analyses of the leaves of tomato plants from the series from which growth data are given in table 1 are shown in table 3. The experimental conditions of this series are described in connection with table 1. Table 3 shows the pronounced effect of lack of manganese on the sugar content of the leaves, the percentage of sugar in the no-manganese plants in some cases being only one-tenth of that of the plants which had received 30 mg. of $MnSO_4$ per culture. The addition of manganese to the manganese-starved cultures brought about a prompt increase in the sugar content. Although these cultures received 90 mg. of $MnSO_4$ as late as April 2, they had already increased in reducing sugar from 0.047 to 0.152 per cent of the fresh weight by April 11.

When the experiments summarized in tables 1 and 3 were concluded, it was decided to try to grow fruits on some low-manganese plants in order to compare them with fruits grown on plants from the other cultures. Many of the plants in the no-manganese cultures had ceased to grow and could not be used for this purpose, but a certain number remained which did not show such pronounced symptoms of manganese deficiency. Blossoms appearing on these plants were carefully pollinated. To the cultures which had previously received 30 mg. of $MnSO_4$ an additional 20 mg. were added on May 6. Individual fruits from these series were sampled and analyzed for their reducing-sugar content. Certain of the fruits were also analyzed for their acid content. An aliquot of an aqueous extract was titrated with $N/10$ NaOH and the acid present calculated as citric acid.

These data are summarized in table 4. It is seen that the fruits from the no-manganese plants are decidedly lower in sugar content than those from the other cultures. Significant differences in acid content are not evident.

DISCUSSION

It is not clear at this time why culture conditions which are similar to those previously used successfully by others without the addition of manganese sul-

TABLE 3. *Reducing sugar and sucrose content of the leaves of tomato plants grown in sand cultures with various amounts of manganese sulphate*

Treatment	Date sampled											
	March 18				March 28				April 11			
	Reducing sugars		Sucrose		Reducing sugars		Sucrose		Reducing sugars		Sucrose	
	% Fresh wt.	% Dry wt.	% Fresh wt.	% Dry wt.	% Fresh wt.	% Dry wt.	% Fresh wt.	% Dry wt.	% Fresh wt.	% Dry wt.	% Fresh wt.	% Dry wt.
Without manganese	0.019	0.19	0.063	0.62	0.012	0.104	0.044	0.39	0.047	0.41	0.088	0.76
With 30 mg. MnSO₄	0.081	0.67	0.106	0.88	0.102	0.80	0.164	1.29	0.213	1.46	0.203	1.39
With 230 mg. MnSO₄	—	—	—	—	0.102	0.79	0.136	1.05	0.124	0.92	0.262	1.93
90 mg. MnSO₄ added to no-Mn cultures April 2	—	—	—	—	—	—	—	—	0.152	1.03	0.225	1.52

TABLE 4. *Summary of data on the reducing sugar and acid content of tomato fruits grown in sand cultures with various amounts of manganese sulphate*

Treatment	Reducing sugars, % of fresh weight					Citric acid, % of fresh weight				
	No. of fruit analyzed	Max. value	Min. value	Average	P.E. average	No. of fruit analyzed	Max. value	Min. value	Average	P.E. average
Without manganese...........	17	2.83	1.39	2.21	±0.075	22	0.73	0.36	0.52	±0.013
With 50 mg. MnSO₄.........	23	3.69	2.56	3.06	±0.036	29	0.60	0.36	0.48	±0.007
With 230 mg. MnSO₄.......	5	3.98	2.70	3.06	±0.23	10	0.60	0.39	0.48	±0.014

phate resulted in manganese deficiency in the case of the experiments here described. It is quite possible that the particular lots of sand and pots used in this work happened to contain much less manganese than is usual for these materials. Several different lots of sand and pots were used, however, with the same results.

McHargue (1926) has reported that qualitative tests showed that manganese-deficient plants in his experiments were lower in sugar than corresponding plants grown in the presence of manganese. He stresses the chlorotic condition of the manganese-deficient plants and emphasizes the importance of manganese in chlorophyll formation. It seems very probable, however, that manganese plays a more fundamental rôle in the formation of sugar. Various nutritional deficiencies result in a lowered chlorophyll content of leaves, and this is not always associated with a decreased sugar content. Low-nitrogen plants are light yellow in color but contain much sugar (Kraus and Kraybill, 1918). The low-manganese plants in the experiments here reported made very little growth and thus did not utilize any large amount of carbohydrate. Even a much lowered chlorophyll content, if it were a question of chlorophyll alone, should have been able to bring about the formation of enough sugar to result in plants not deficient in sugars.

It is of interest to compare the results obtained with manganese-deficient plants with studies of plants grown under conditions of starvation with respect to some other necessary element. Low-nitrogen and low-phosphorus plants, contrary to low-manganese plants, are high in reducing sugars and sucrose (Eckerson, 1931; Kraybill and Smith, 1924; Kraybill, 1930; Kraus and Kraybill, 1918). In the case of potassium-deficient plants, carbohydrates frequently accumulate, although in the later stages plants are low in carbohydrates (Nightingale, Schermerhorn, and Robbins, 1930).

SUMMARY

1. Wheat, corn, lettuce, cabbage, rye, barley, tobacco, and tomato plants were grown under manganese-deficient conditions.

2. The manganese necessary for the growth of tomato plants could be added directly into the stem of the plants. It was thus possible to grow manganese-deficient plants in the same pot with plants receiving manganese.

3. Manganese-deficient plants were much lower in sugar than corresponding plants which had received a small amount of manganese. Tomato fruits grown on low-manganese plants were lower in sugar content than fruits from plants grown in cultures to which manganese had been added.

4. The results indicate that manganese plays an important rôle in sugar formation and sugar metabolism, either directly or indirectly.

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COPPER AND IRON IN THE TRACHEAL SAP OF DECIDUOUS TREES

J. P. BENNETT AND JACOB OSERKOWSKY

Copper has been shown to be of importance in plant metabolism (Lipman and MacKinney, 1931; McHargue and Calfee, 1931; Sommer, 1931). In animals it has been found to be related to the functioning of iron (Underhill, Orten, and Lewis, 1931). The work reported in this paper was undertaken as part of an attempt to get information which might suggest whether or not there is any functional relation between the two elements in plants. It was suggested by the occurrence within a single small pear orchard of chlorosis curable only by the administration of iron, and of a disease of pear trees curable only by the administration of copper to the trees (Smith and Thomas, 1928). There are presented here only data on the content of copper and iron, and total electrolytes of the tracheal sap of certain fruit trees, especially pear trees.

Tracheal sap was obtained according to the gas displacement method (Bennett, Anderssen, and Milad, 1927). The sap was evaporated in silica crucibles, ashed, and the ash dissolved in HCl. Iron and copper were determined colorimetrically, iron by the thiocyanate method, and copper according to Elvehjem and Lindow (1929). An indication of the total electrolyte content of the sap was obtained by determining its electrical conductance. Some of the conductance measurements were made by Mr. V. W. Smart, to whom acknowledgment is due.

Single Bartlett pear trees growing on a clay, loam, slightly acid soil were dug at about monthly intervals. The branches of each tree were segregated in groups according to age, and the tracheal sap was extracted from each group separately. Figure 1 shows the seasonal variation in the concentration of copper, iron, and total electrolytes in the sap of these groups of branches. The highest concentrations of all three constituents occurred in the early spring except in the youngest branches. By early summer the concentration of copper and iron had fallen to a much lower value; and in the autumn a second maximum of concentration occurred, giving way to a winter level approximating that of summer. An exception to this general statement of seasonal levels of concentration of copper and iron occurred in the copper concentration in the winter sap of the three-year-old branches, which is very high, and in the apparent absence of autumn maxima in the curves for the sap from five-year-old maxima. The specific conductance shows the same seasonal trends as the copper and iron concentrations in general but with relatively
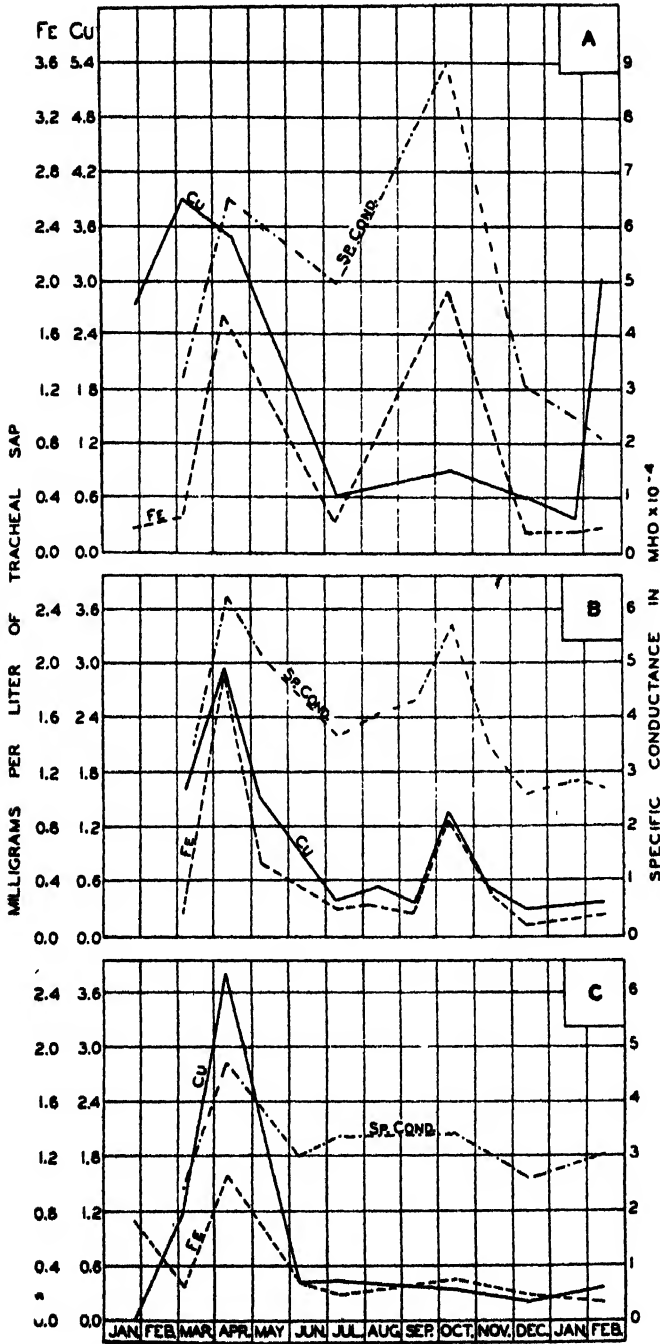
Fig. 1. Specific conductance, copper and iron content in tracheal sap of 3-year-(*A*), 4-year-(*B*), and 5-year-(*C*)-old Bartlett pear branches.
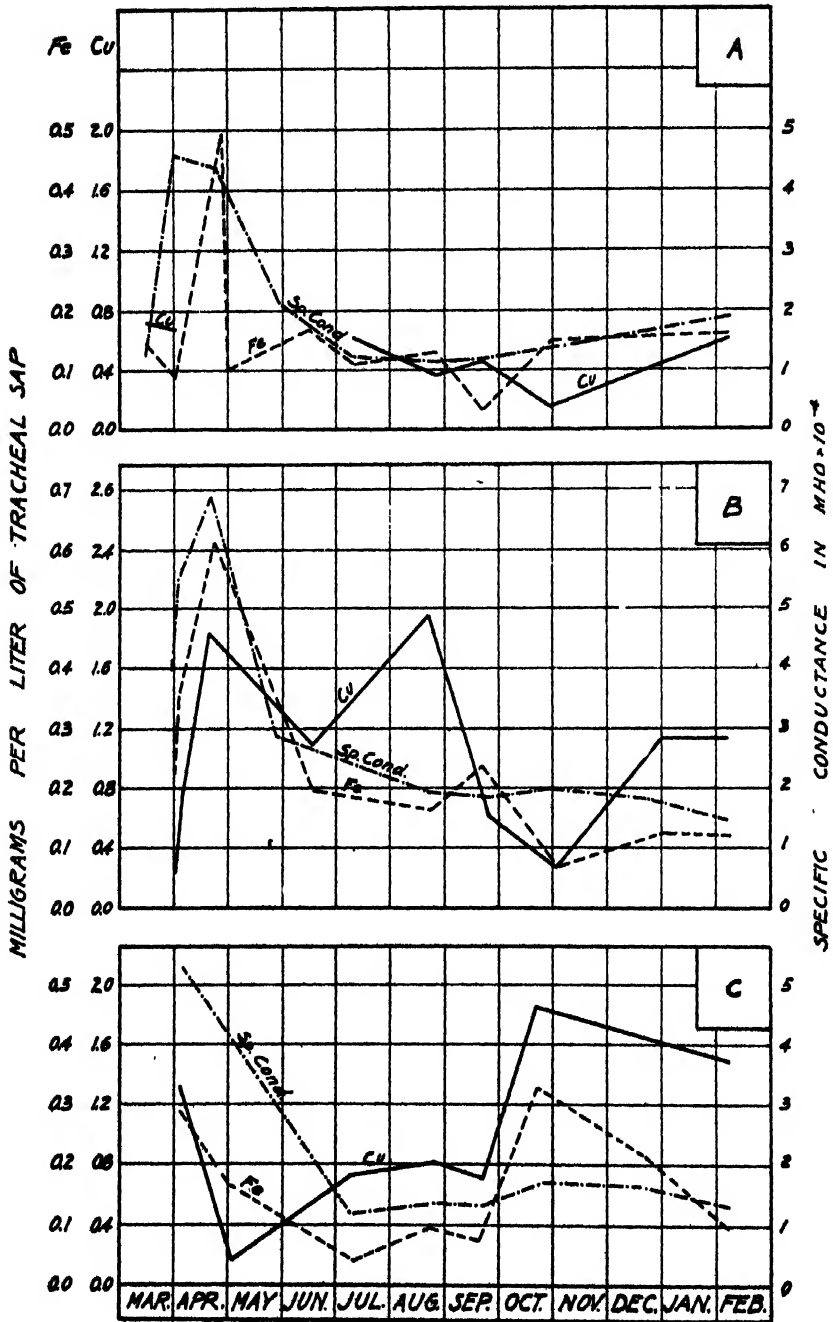
Fig. 2. Specific conductance, copper and iron content in tracheal sap of chlorotic branches (*A*), green branches from trees grown on calcareous soil (*B*), and green branches from trees on non-calcareous soil (*C*).

less difference from the spring maximum in summer, autumn, and winter. Sufficient evidence is not available to justify an attempt to explain the various changes in concentration; and nothing can be said concerning the failure of the autumn maxima to appear in the sap from the five-year-old branches. It appears to be of possible significance that the autumn maxima, aside from copper, are higher the younger the branches. The spring maxima in concentration coincide with the swelling and opening of buds; and the subsequent decrease occurs at the time of most rapid growth. The changes in concentration of the sap are apparently closely related to the state of activity within the plant.

In figure 2 is shown the concentration of iron, copper, and total electrolytes in the sap obtained from three series of Hardy pear branches. The branches were not separated according to age; most branches used contained wood from three to six years old. In part *A* the branches were from chlorotic trees growing in a highly calcareous adobe soil; in part *B* they were from green trees growing in a moderately calcareous adobe soil; and in part *C* they were from green trees growing on an alluvial sandy noncalcareous loam.

A spring maximum of concentration of iron, copper, and total electrolytes is indicated in the sap from the three series, although complete data are not available in all cases. In these sets of branches the specific conductance of the sap, after the spring period, fell to a much lower level and remained lower through the remainder of the year than in the data shown in figure 1. The trends of the copper and iron curves do not agree closely with those shown in figure 1 except in the spring period; during the remainder of the year the trend is much less uniform, and an autumn maximum is clearly evident only in part *C*.

Considering the data presented in both figures 1 and 2, there was in general a strong similarity of trend between the copper, iron, and total electrolyte concentration curves, despite the occurrence of some exceptions which may have been due to errors of determination. The general features of the curves are the same: a strong rise in all three constituents in the spring, a subsequent decrease, sometimes a secondary autumn maximum, and a final winter level about the same as that of summer. Iron and copper trends showed a closer similarity to each other than did either to total electrolytes. The similarity of trend of total electrolytes and iron and copper could not be attributed to the iron and copper ions, since the fraction of current carried by these ions, even if it were assumed that all the iron and copper present was in ionic form, was insufficient to account for more than a small portion of the observed conductance. The relation between the seasonal trends of the curves suggests that the concentration of iron and copper salts is governed by the same factors that determine the concentration of other electrolytes in the tracheal sap. One may infer that the most important of these factors are: The rate of absorption from the soil, which is dependent upon

the availability of iron, copper, and other salts in the soil and the condition of the absorbing root system; and the rate of absorption and utilization of the various salts by the tissues. During the active growing season the salt content of the sap would be chiefly the resultant of these two processes. In the early spring, however, there appears to be a third important factor. The large and rapid increase of copper, iron, and total electrolytes in the tracheal sap appears to be more probably due to release from the tissues than to absorption from the soil. There occurs at the same time a corresponding increase in organic substances, especially sugars (Anderssen, 1929), which can come only from the tissues themselves. Release of electrolytes and sugars from the living cells of xylem would involve changes in permeability of the cells or changes in concentrations of substances within the cells, or both.

In table 1 are presented data showing the copper and iron content of the tracheal sap of several kinds of fruit trees, mostly in the spring. These data further illustrate the order of magnitude of the copper concentration. They show that copper is usually higher than iron in the sap; the same holds true also for the data shown in figures 1 and 2.

TABLE 1. *Iron and copper in tracheal sap of various fruit trees*

Plant	Part of tree	Date collected	Fe in sap (p.p.m.)	Cu in sap (p.p.m.)
Bartlett pear	Large roots	Feb. 11	0.2	0.7
	"	March 4	0.4	3.0
	"	April 8	0.4	1.0
	"	Nov. 10	0.2	0.4
	"	Dec. 9	0.5	0.6
	Medium sized roots	Feb. 11	0.4	0.6
	"	March 4	0.3	0.9
	"	Nov. 10	0.3	0.9
Peach	Branches	March 1	0.4	1.5
	"	March 4	0.2	0.5
	"	March 6	0.4	1.1
	"	March 7	0.3	1.0
	Roots	March 1	0.5	0.6
	"	March 4	0.7	1.1
	"	March 7	0.5	0.4
Apricot	Branches	March 13	1.5	3.7
	"	March 21	1.5	2.1
	Roots	March 21	1.2	1.3
Almond	Branches	April 1	0.4	0.9
	Roots	April 1	0.3	1.1
Quince	Branches	April 3	0.3	1.4
Prune	Branches	March 23	0.4	—
	"	March 30	0.3	1.0
Cherry	Branches	March 31	1.1	1.0

The presence of copper in the tracheal sap, the response of the diseased trees mentioned earlier to copper, and the work cited indicate that copper plays an important rôle in plant metabolism. But there is nothing in this

that necessarily indicates any special relationship of copper to the functioning of iron. The similarity of the seasonal trends of the copper and iron curves also cannot be considered as such an indication, since the same trend is shown by total electrolytes. The parallelism between copper and iron in the sap therefore seems to be purely incidental.

SUMMARY

1. The annual changes in iron, copper, and total electrolyte content of the tracheal sap from branches of pear trees were found to be generally similar.

2. The trend of changes was: a rapid rise in concentration in early spring from a lower winter level; a subsequent rapid fall in late spring to a summer level approximating that of winter; a continuance of the summer low level into winter with or without a secondary autumn rise and fall.

3. The parallelism shown in the curves indicates that the iron, copper, and total electrolyte concentrations in the sap are controlled by the same factors.

4. The tracheal sap from branches or roots of several varieties of fruit trees usually contained more copper than iron.

5. No special relation of copper to the functioning of iron is indicated by the data.

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COMPARATIVE ANATOMY OF THE WOODS OF THE MELIACEAE, SUB–FAMILY SWIETENIOIDEAE

A. J. Panshin

INTRODUCTION

The purpose of this investigation is to present detailed information on the comparative anatomy of the woods of the Meliaceae, sub-family Swietenioideae, to determine whether it is feasible to separate the genera comprising this group on the basis of wood anatomy, and finally, to ascertain whether they maintain the same relationship when thus classified as in the accepted natural classification.

In flowering plants experience has taught that classification based on the morphology and anatomy of the flower, that is, " natural classification," best indicates botanical affinities, but in such a classification little or no attention has been paid in the past to the wood anatomy of the plants concerned. The majority of investigators contend that very little parallelism can be detected in the evolution of reproductive and vegetative organs (including wood) and that, therefore, the two cannot be correlated for the purpose of classification. Bailey (1), for example, concludes that often the primitive types of vascular structures are retained in some representatives of florally highly organized groups and vice versa, and furthermore, that frequently similar salient vascular structures occur in widely separated genera and orders.

But even though no parallelism exists in the evolution of floral parts on one hand, and of woody tissue on the other, a detailed investigation of wood anatomy in such natural groups as families and sub-families may serve a useful purpose. It may, as Kribs (5) has pointed out, " bring to light many important facts which will be of value to systematic botanists in clearing up doubtful points." Certainly, in any case, it will serve to establish on a firmer and more logical basis the classification and identification of woods of these natural groups, always provided a sufficient number of authentic and representative wood specimens are available for study.

The New York State College of Forestry at Syracuse, New York, was very fortunate in obtaining a very large and varied collection of authentic wood samples of African Swietenioideae (of the genera *Khaya, Entandrophragma, Pseudocedrela,* and *Lovoa*) ; sufficient material of the allied genera *Swietenia, Soymida,* and *Chukrasia* was also available in the collections of the Department of Wood Technology at the same institution.

THE MAHOGANY FAMILY (MELIACEAE)

The Mahogany family consists of 40 genera and about 800 species of trees, shrubs, or rarely woody herbs, widely distributed in the tropical and sub-tropical regions of both hemispheres, a few species extending into the temperate zones. Meliaceous plants are characterized by alternate or occasionally opposite, pinnately compound (rarely palmately compound or simple) leaves with opposite or alternate, usually entire leaflets; regular, perfect, or polygamo-dioecious flowers in axillary or terminal panicles, racemes, spikes, or occasionally in umbels; and capsular, baccate, or drupaceous fruits, the first bearing winged or unwinged seeds.

The woods of this family range through shades of red, reddish-brown, or less frequently chocolate-brown or white, and exhibit all degrees of sheen from lustrous to dull. The weight and hardness likewise vary between wide extremes; the genus *Cedrela,* for example, includes a number of very soft, light woods with specific gravities between 0.30 and 0.65, while the other extreme is represented by the hard and heavy woods of *Soymida* species which, when oven-dry, will usually sink in water (Sp. Gr. 1.0–1.2). Some of these woods are pleasantly scented, relatively few (such as the Onionwood of Australia, *Owenia cepidora* F. Muel.), have unusual aromas, while many others are devoid of odor or rapidly lose their scent when exposed to the air. The grain and texture also exhibit wide variation, but there is a decided tendency toward interlocking of the grain, with corresponding ribbon and roey figures in quarter-sawn lumber. Growth rings are frequently distinguishable, but most of the woods are of the diffuse porous type; in fact, ring porous woods are found in only two genera. The vessels range from very small to large and occur either solitary or in short radial groups, rarely in small nests; they generally contain copious deposits of reddish-brown or black gum, and in some instances chalky inclusions are also present; the vessel perforations are simple throughout, and the inter-vessel pits are mostly small and often with confluent apertures. The fibers are usually fine and thin-walled, and are often septate and gelatinous. Parenchyma is relatively abundant around the vessels, and zonate and metatracheal parenchyma in varying amounts are not infrequently present. The rays may be either very narrow and indistinct in the transverse section or comparatively wide and visible with the naked eye; they vary in width from 1 to 10 cells, and are either of the homogeneous type or decidedly heterogeneous. Ripple marks feature a few of these woods. Lysigenous gum canals, possibly of traumatic origin, are present in many cases.

The economic importance of the Meliaceae centers primarily on timber production, since many of these trees produce valuable ornamental woods sought by the trade, among which the following deserve mention: genuine Mahogany from *Swietenia* spp.; Spanish Cedar, the product of *Cedrela odorata* Linn..; the Toon of India from *Cedrela Toona* Roxb.; the "African Mahoganies," produced by the genera *Khaya* and *Entandrophragma.* Several

drugs of minor importance are also contributed by this group, among them the extract "Azedarach," obtained from the various plant parts of *Melia Azedarach* Linn. "Oleum Cedrelae," the cedarwood oil of druggists, is extracted from the wood of various *Cedrela* species and is used in the perfume industry.

SUB-FAMILY SWIETENIOIDEAE: DISTINGUISHING BOTANICAL CHARACTERS AND GENERA INCLUDED

Harms (3) divided the Meliaceae into three sub-families—namely, the Cedreloideae, Meliodeae and Swietenioideae. In this classification the Swietenioideae are featured by the following characters: stamens connate in the tube; seeds winged; ovary with several to many ovules in each cell.

As originally understood by Harms, the Swietenioideae included 8 genera—viz., *Chukrasia, Entandrophragma, Elutheria, Khaya, Lovoa, Pseudocedrela, Swietenia,* and *Soymida.* Later, he (4) added to this group a West African genus, *Wulfhorstia,* but this has since been combined by Sprague with the genus *Entandrophragma.* No material of *Elutheria* was available for the study, and hence, in this paper the Swietenioideae are treated as consisting of seven genera. Four of these—namely, *Entandrophragma, Khaya, Pseudocedrela,* and *Lovoa*—are restricted to tropical Africa (largely to the West Coast and Central Africa), and together embrace about thirty species. *Swietenia,* with 3–5 species, is limited to the West Indies, Mexico, and Central America. *Chukrasia* is widely distributed in the Orient from the Western Peninsula of India to Southern China, and includes one, possibly two, species. Finally, one species of *Soymida* is indigenous to India, and a second species is said to occur in tropical Africa.

The above summary will serve to indicate that in point of number of genera and species the center of distribution of the Swietenioideae is in West and Central tropical Africa. The botanical characters which serve as a basis for the separation of the genera are enumerated in the following key:

Key to the genera of Swietenioideae on the basis of morphological characters

1. Seeds winged above or below or at both ends 2
1. Seeds winged all around ....*Khaya*
    2. Seeds winged above or below 3
    2. Seeds winged at both ends ....*Soymida*
3. Fruit a 5-celled, 5-valved (very rarely 4–6 celled, 4–6 valved) capsule 4
3. Fruit a 3- or 4-celled, 3- or 4-valved capsule 6
    4. Capsule oblong or elongated; seeds winged below 5
    4. Capsule ovoid; seeds winged above ....*Swietenia*
5. Staminal tube connected to the stalk-like disk by longitudinal ridges; leaflets entire
                                                                      Entandrophragma
5. Staminal tube not connected to the disk by ridges; leaflets undulately toothed
                                                                      Pseudocedrela
    6. Capsule ovoid, 3-celled, 3-valved, woody; seeds winged below ....*Chukrasia*
    6. Capsule narrowly oblong to quadrangular, 4-celled, 4-valved, membranous; seeds
       winged above ....*Lovoa*

AMERICAN SWIETENIOIDEAE

The American representatives of the Swietenioideae are restricted to the genera *Swietenia* Jacq. and *Elutheria* M. Roem, the authenticity of the last being questionable; the genuine mahogany of the trade is produced by the genus *Swietenia,* treatment of which follows.

Swietenia Jacq.

Botanical description.

Large tropical trees, frequently over 100 ft. in height, and 4 to 5 ft. in diameter, with a tall, straight, cylindrical bole clear of branches for 40 to 60 ft. and often buttressed to a height of 10–15 ft.   Leaves alternate, pinnate, with opposite or occasionally sub-opposite or alternate leaflets.   Flowers perfect, pentamerous, paniculate.   Fruit a 5-celled (rarely 4-celled), 5-valved (rarely 4-valved), woody capsule; seeds 10–14 in each cell, winged above.

Range and distribution.

Swietenia is distributed throughout the West Indies, Bermuda, the Bahamas, keys of southern Florida, Mexico, Central America, Colombia, Venezuela, Ecuador, and Peru.   Six species have been described, but Blake (2) in his revision of the american mahoganies, recognizes only five—namely, *S. mahagoni* Jacq., confined to the West Indies, Bermuda, the Bahamas, and the southern keys of Florida; *S. macrophylla* King, distributed throughout southern Mexico, the eastern coast of Central America into Colombia, and, according to Williams (9), in Peru; *S. humilis* Zucc., ranging through the western coast of Mexico and Central America; *S. cirrhata* Blake, reported by the author from the Pacific Coast of Mexico, from Sinaloa, Michoacan, Oaxaca, and El Salvador; and finally, *S. candollei* Pittier, limited to Venezuela.   Recently, however, Standley (8) has pointed out that *S. cirrhata* is not tenable, since it is indistinguishable from *S. humilis.*   Likewise, it has been suggested that *S. candollei* is synonymous with *S. macrophylla.*

General description of the wood.

Mahogany wood ranges in color from very light pink to a dark reddish-brown, and possesses a pronounced satiny or golden lustre.   That of *S. mahagoni* is generally darker, heavier, and more highly figured than timber from the species growing on the mainland.   The lightest and softest wood from the mainland is known in the trade as baywood mahogany.   It is said to be produced by *S. macrophylla,* while the harder and more colored variety is thought to be a product of *S. humilis.*   In general, however, the woods of the *Swietenia* species cannot be separated anatomically with any degree of certainty, although, according to Record and Mell (7), considerable variation exists in their mechanical and physical properties, depending upon the character of the soil, general conditions of growth, and the species.   According to these authors, these differences are so great that experts can distinguish mahogany from various regions at a glance.

Swietenia mahagoni Jacq.

General Characteristics of the wood:

Sapwood yellowish-white; heartwood very pale pink to dark reddish-brown, the freshly cut lumber darkening with exposure; with a satiny or golden lustre; odorless; soft to hard; light to heavy (Sp. Gr. 0.35–0.85); medium fine textured; straight to inter-locked grained, in the latter case frequently showing a variety of figures on the quarter. Growth rings distinct, demarcated by a line of terminal parenchyma. Pores scarce to fairly numerous, evenly or unevenly distributed and then sparse or frequently absent from the outer portion of the ring, solitary or in short radial groups, small to fairly large, the latter generally visible with the naked eye, frequently occluded with dark reddish-brown infiltration, or with white chalky deposits; vessel lines distinct, plugged with reddish-brown or white infiltration. Terminal parenchyma very conspicuous as light, concentric lines at more or less regular intervals; paratracheal parenchyma in-conspicuous. Gum canals frequently present, arranged in well defined concentric rows (x),[1] visible with the naked eye in the transverse and longitudinal sections. Rays fairly numerous, uniform in width, barely visible or indistinct with the naked eye; ray fleck low but fairly conspicuous, usually lighter than the background. Ripple marks generally present, 20–25 per cm.

Minute anatomy:

Growth rings delimited by a 2–15 seriate band of terminal parenchyma (or a wider band of this sort containing gum canals), and frequently in addition by denser fibrous tissue toward the outer margin.

Vessels solitary or in radial rows of 2–10, occasionally in tangential groups of 2–3 or in nests, encircled by a 1–3 seriate sheath of parenchyma, frequently interrupted by fibers and rays contiguous to the vessel, 4–12 (av. 8) per mm.[2]; orifices round or oval, the largest 110–170 microns in diameter; vessel segments 150–500 microns long, truncate or rarely tailed; lateral walls 5–12 microns thick; perforations simple, round, or oval, horizontal or nearly so; inter-vessel pits very numerous, minute, 2–3 microns in diameter, rounded or irregularly polygonal, the apertures of 2-many often confluent; pits leading to contiguous rays numerous to each cell, similar to those of the inter-vessel type; red-dish-black infiltration and chalky-white deposits abundant.

Fibers medium fine, thin- to moderately thick-walled toward the outer portion of the seasonal increment, inconspicuously arranged in radial rows, septate or non-septate, gelatinous or non-gelatinous, rounded or angular and then frequently rectangular in the transverse section, 18–24 microns in diameter, 600–1600 microns long; walls 2–6 microns thick; inter-fiber pits small, round, simple for the most part; dark red infiltration occasional.

Parenchyma (*a*) paratracheal, (*b*) metatracheal, and (*c*) terminal, mostly in cambiform rows along the grain, the units of which are frequently further subdivided into loculi containing solitary crystals: (*a*) *paratracheal* parenchyma in a 1–3 seriate sheath which is often interrupted by rays and fibers contiguous to the vessel; (*b*) *meta-tracheal* parenchyma sparse to abundant, occurring as solitary cells or in small groups; (*c*) bands of *terminal* parenchyma 2–15 seriate or wider, and then frequently including 1-many rows of gum canals filled with dark-red infiltration; cells of all types of paren-chyma rounded or rectangular in the transverse section (frequently flattened to conform to the vessel wall in the case of paratracheal parenchyma), about twice as wide as the fibers, 28–50 microns wide, 50–120 microns long; reddish-brown infiltration sparse to copious; crystals occasional.

Gum canals, when present, arranged in tangential rows (1-many), imbedded in terminal parenchyma.

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[1] Throughout this paper x designates the cross or transverse section, t the tangential, and r the radial sections.

Rays 4–7 per mm., 1–6 (mostly 3–4) seriate,[2] intergrading in width but the uniseriate and biseriate rays neither abundant nor prominent, generally more or less regularly storied, heterogeneous, up to 90 microns (mostly 50–60 microns) wide, up to 35 cells and 1200 microns (mostly 15–25 cells and 300–600 microns) in height; "upright" cells solitary or rarely 2–3 at each end of the ray (*t*), 30–60 microns high, 24–30 microns wide, 50–60 microns long; "horizontal" cells round to oval, frequently considerably smaller in the core and larger on the flanks of the multiseriate portion of the ray, 10–30 microns high, 10–20 microns wide, 100–200 microns long; pits leading to contiguous vessels similar to the inter-vessel type, numerous to each cell, 3–4 microns in diameter, orbicular for the most part; reddish-brown infiltration abundant; rhomboid crystals present in the "upright" cells.

Ripple marks, when present, traceable to storied rays.

Material: Syracuse Nos. 159/3494,[3] St. Croix; 42/911,[3] Porto Rico; 159/3566,[3] Collegio de la Salle, Habana, Cuba; 1-M [3] and 2-M,[3] San Domingo, per C. D. Mell, identified by Blake, 1930; 159/2173 [3] U. S. Nat. Mus. No. 705; 42/955,[3] Java; 159/1871, Java; [3] U. S. Nat. Mus. No. 1041,[3] Jamaica; also numerous trade samples from Cuba, Porto Rico and Jamaica.

Swietenia macrophylla King

The wood of this species is generally lighter in color, softer, lighter in weight (Sp. Gr. 0.4–0.65), and slightly coarser textured than that of *S. mahagoni*.

Minute anatomy:

Similar to that of *S. mahagoni* with the following exceptions: *vessels* 180–230 microns in diameter; *fibers* 18–30 microns in diameter.

Material: Mell No. 302,[3] Oaxaca, Mexico, identified by Blake; U. S. Nat. Mus. No. 1074,[3] Guatemala, Blake No. 7866; Syracuse Nos. 159/3077; [3] 42/954,[3] Java; 159/3378, Br. Honduras; 41/16; trade samples Nos. 320–330.

Swietenia humilis Zucc.

Not exhibiting as wide a range in weight as *S. mahagoni* but heavier than *S. macrophylla*. Sp. Gr. 0.55–0.74. Indistinguishable anatomically from the wood of *S. mahagoni*.

Material: Mell No. 300,[3] San Blas, Nayaret, Mexico; Mell No. 301,[3] San Pedro, Chiapas, Mexico, both identified by Dr. J. F. Blake; U. S. Nat. Mus. 2314,[3] Sinaloa, Mexico.

INDO-MALAYAN SWIETENIOIDEAE

The Swietenioideae are represented by two genera in the Indo-Malayan region, *Chukrasia* A. Juss., embracing one or possibly two Indo-Malayan species, and *Soymida* A. Juss., with one species, *S. febrifuga* A. Juss., in India and possibly a second species in tropical Africa.

Chukrasia A. Juss.

Botanical description.

Tall trees, up to 6 ft. in diameter, with a straight, cylindrical bole, clear of branches for some 30 ft. Leaves pinnate with 10–24 usually alternate leaflets. Flowers white, 4- or 5-merous, in terminal panicles. Fruit a 3-celled, 3-valved, woody capsule. Seeds numerous, flat, winged below.

[2] One trade sample examined showed rays 1–7 (mostly 5) seriate.
[3] Authentic wood samples.

Size and distribution.

Chukrasia is endemic to the tropical and sub-tropical regions of the Orient, and includes the type species *C. tabularis* A. Juss., and the well marked variety, *C. tabularis* var. *velutina* Roem. which is recognized by some authorities as a separate species. According to Pearson and Brown (6) the original range of this species is in doubt. It now extends from the Western Peninsula of India, eastward into Ceylon, Burma, the Malay Peninsula, Borneo, Cochin-China, and Southern China.

Description of the wood.

Since *Chukrasia* includes but one species, see under "Species" for the "General Characteristics" and "Minute Anatomy" of this wood; data on the last were obtained largely from "The Commercial Timbers of India," by Pearson and Brown (6).

●   *Chukrasia tabularis* A. Juss.

General description of the wood:

Sapwood pale-yellowish or brownish-white, grading into the heartwood; heartwood yellowish-red to red, darkening on exposure to yellowish or reddish-brown; with a satiny lustre; tasteless and odorless; medium hard; light to moderately heavy (Sp. Gr. approx. 0.62); medium fine textured; frequently interlocked grained. Growth rings visible with the naked eye, inconspicuous, demarcated by a narrow line of terminal parenchyma, and sometimes in addition by denser fibrous tissue in the outer portion of the ring. Pores sparse to fairly numerous, quite evenly distributed, mostly solitary and in short radial groups, occasionally nested, small to medium large, barely visible with the naked eye, open or occluded with yellowish-white deposits; vessel lines inconspicuous. Terminal parenchyma visible with the naked eye, appearing as a pale brown line; other types of parenchyma indistinct, even with a lens (10 ×). Rays fairly numerous, uniform in width, fine and indistinct with the naked eye; ray fleck fine, darker than the background. Ripple marks present but obscure.

Minute anatomy:

Growth rings delimited by a 2–5 seriate band of terminal parenchyma and frequently in addition by a denser fibrous tissue toward the outer margin.

Vessels quite evenly distributed, solitary and in radial rows of 2–3, occasionally nested, surrounded by a uniseriate interrupted sheath of paratracheal parenchyma, 5–25 per mm.^{2}, the largest 150–165 microns in diameter; vessel segments 170–500 microns long, truncate or abruptly or attenuate-tailed; lateral walls 2–4 microns thick; perforations simple, nearly horizontal to somewhat oblique; inter-vessel pits numerous, narrowly elliptical, the long diameter 3–6 microns, the apertures of 2-many frequently confluent; pits leading to contiguous rays numerous to each cell, similar to those of inter-vessel type; pale yellowish-white granular deposits frequent.

Fibers fine, thin- to thick-walled, occasionally septate, 28–32 microns in diameter, very variable in length (310–1650 microns); inter-fiber pits largely confined to the radial walls, bordered.

Parenchyma (a) paratracheal, (b) metatracheal, and (c) terminal: (a) *paratracheal* parenchyma sparse, forming a uniseriate, interrupted sheath and frequently restricted to 2–3 cells to a vessel; cells flattened, the long diameter 45–50 microns; (b) *metatracheal* parenchyma extremely sparse; cells solitary; (c) bands of *terminal* parenchyma distinct, continuous, 2–5 seriate; cells of "b" and "c" parenchyma 20–32 microns in diameter; light yellowish-brown gummy deposits frequent in all types of parenchyma; crystals rare.

Rays 5–8 per mm., of nearly uniform width, 1–6 (mostly 3–4) seriate, frequently obscurely storied, homogeneous or nearly so, the largest 60–65 microns wide and up to 35 cells and 800 microns in height; pits leading to vessels numerous to each cell, 3–4 microns in diameter, the apertures of 2-many frequently confluent; light yellowish-brown infiltrations copious; crystals not observed.

Ripple marks obscure, traceable to the rays.

Material: Gamble specimen, No. 5842;[4] Cockrell No. 25, *C. tabularis* var. *velutina*;[4] also trade specimens.

Soymida A. Juss.

Botanical description.

Moderately large to large, semi-evergreen trees, with a bole up to 10 ft. in diameter, clear of branches for 12 to 14 ft. above the ground, with grayish-brown bitter bark. Leaves alternate, paripinnate, with 3–6 pairs of opposite leaflets. Flowers bisexual, pentamerous, subtended by triangular bracts, in large terminal panicles. Fruit a 5-celled, 5-valved capsule. Seeds numerous in each cell, flat, winged at both ends.

Size and distribution.

Two species have been described, *S. febrifuga* A. Juss., indigenous to the dry forests of the Western Peninsula and Central India, and extending northward to Merwara, the Mirzapur Hills, and Chota Nagpur, and *S. roupalifolia* Schw., which is reported to occur in tropical Africa, a statement, which, however, requires further confirmation.

General description of the wood.

Since material of the African species was not available for study, the data compiled on the gross and minute anatomy of Soymida wood apply only to the Indian species, *S. febrifuga* A. Juss.; for description of this wood see under "Species." "The Commercial Timbers of India," by Pearson and Brown (6), was consulted in assembling this information.

Soymida febrifuga A. Juss.

General characteristics of the wood:

Sapwood small, whitish; heartwood ranging through shades of dark blood-red or darker purple to rich reddish-brown, or chocolate-brown, frequently with lighter streaks; dull; odorless and tasteless; hard; very heavy (Sp. Gr. 1.0–1.2); coarse textured, irregular and twisted but not interlocked grained. Growth rings indistinct with the naked eye, visible with the lens (10 ×), delineated by a faint narrow line of terminal parenchyma. Pores scarce to fairly numerous, unevenly distributed and frequently most numerous in the early portion of the ring, solitary or in short radial groups, small to medium large, scarcely visible with the naked eye, occluded with brown infiltration, or rarely with chalky deposits; vessel lines distinct, nearly black through infiltration. Brownish lines of terminal parenchyma scarcely visible with the naked eye, concentric, 4–40 per inch; paratracheal parenchyma sometimes visible with the naked eye. Rays fairly numerous, barely visible or indistinct with the naked eye; ray fleck of the same color as the background, inconspicuous. Ripple marks absent.

[4] Authentic specimens.

Minute anatomy:

Growth rings delimited by a 1–7 seriate line of terminal parenchyma.

Vessels unevenly distributed and most numerous in the early portion of the ring, solitary, in radial groups of 2–6, or rarely nested, 0–46 per mm.[3] the largest 200–215 microns in diameter; vessel segments 265–375 microns long, truncate; lateral walls 6–12 microns thick; perforations simple, horizontal, or nearly so; inter-vessel pits numerous, minute (3–4 microns in diameter), orbicular or angular, the apertures of 2-many frequently confluent; pits leading to contiguous rays numerous to each cell, similar to those of inter-vessel type; deposits of brown gum very abundant, occluding most of the vessel segments.

Fibers rather fine, thick-walled or occasionally thin-walled, not in distinct radial rows, frequently septate, rounded in the cross section, 24–28 microns in diameter, 335–1420 microns long; walls 3–9 microns thick; inter-fiber pits simple by reduction, with slit-like oblique orifices; brown gummy infiltration abundant, occluding many fiber lumina.

Parenchyma abundant, (*a*) paratracheal, (*b*) metatracheal, and (*c*) terminal, in cambiform rows along the grain: (*a*) *paratracheal* parenchyma in a 1-several seriate, sometimes interrupted sheath; cells flattened to conform to the vessel wall, with maximum diameter of 45–60 microns; (*b*) *metatracheal* parenchyma scattered or several cells contiguous to a ray, occasionally crystalliferous; (*c*) *terminal* parenchyma in ragged, 1–7 (mostly 3–5) seriate, concentric lines, which apparently do not indicate annual increments; cells of "b" and "c" parenchyma 25–40 microns in diameter; copious brown gummy infiltration occluding all types of parenchyma; crystals sometimes present, embedded in gum.

Rays 3–7 per mm., unstoried, heterogeneous, of two sizes: (*a*) *large rays* numerous, 5–10 (mostly 6–8) seriate, up to 160 microns in width, 100 plus cells and 2000 plus microns in height; (*b*) *small rays* sparse, 1–2 seriate; up to 10 cells and 200 plus microns in height; "upright" cells usually solitary at each end of the ray (*t*); pits leading to vessels numerous to each cell, oval to elliptical, 3–4 microns in diameter, with evident border; brown gummy infiltration copious; white deposits occasional; crystals rare, embedded in gum.

Material: Gamble Specimen No. 5751;[5] Hand Specimen Dehra Dun, No. 1113; Syracuse No. 42/635, Dehra Dun, India.

AFRICAN SWIETENIOIDEAE

Investigation of the woods of the African Swietenioideae has been made possible through extensive collections made in person by Mr. B. A. Krukoff in five of the West Coast colonies—namely, the Gold Coast, the Ivory Coast, Nigeria, French Cameroon, and French Gabon. In these rich collections the wood samples in every instance are backed by herbarium material from identical trees, the latter identified by Drs. H. Harms and J. Mildbraed of the Berlin Botanischer Garten und Botanisches Museum. The African Swietenioideae are represented by four genera—namely, *Entandrophragma* C. DC., *Khaya* A. Chev., *Lovoa* Harms, and *Pseudocedrela* Harms, all confined to the West Coast and Central Tropical Africa. Of these, *Khaya* and *Entandrophragma* produce the bulk of the "African Mahogany," and *Lovoa* contributes the "Tiger Wood" or "African Walnut" of the trade. The wood of *Pseudocedrela,* although of good quality, is not available in large quantities and is used only locally.

[5] Authentic specimen.

Note should be made at this place of the fact that the scientific names used in the text are those affixed to the herbarium specimens by Drs. Harms and Mildbraed, and that, in the case of the *Khaya* and *Entandrophragma* species, these in certain instances are dissimilar to those accepted by the British and French systematists. However, since these departures in nomenclature will undoubtedly be incorporated into the forthcoming revision of the African Meliaceae by Dr. H. Harms, it has seemed advisable to employ them in this paper.

Entandrophragma C. DC.

Botanical description.

Large to very large forest trees reaching upwards to 120 ft. in height and and 3–8 ft. in diameter, with straight cylindrical trunks clear of branches for 80 or more feet. Leaves alternate, paripinnate, with opposite or sub-opposite, entire-margined leaflets. Flowers pentamerous, in wide cymose panicles. Fruit an elongated, 5-celled, 5-valved, woody capsule. Seeds numerous, winged above.

Size and distribution.

Entandrophragma is restricted to tropical Africa, the center of distribution occurring on the West Coast. Some 20 species have been described, but many of these are only imperfectly known and cannot be accorded specific rank. Of the species described in this report, *E. angolense* C. DC., with its numerous varieties, is widely distributed in West Africa, ranging from French Guinea to Angola and eastward to Uganda: throughout its range it is found scatteringly in the " closed forest " of the dry and moist types, but more frequently in the latter. *E. candollei* Harms extends from French Guinea to Angola, attaining its maximum frequency and development in the Cameroons. *E. cylindricum* Spr. ranges from the Ivory Coast to Angola and thence eastward into Uganda, reaching its optimum development in Nigeria. Finally *E. utile* Spr. also extends from the Ivory Coast to Angola and eastward to Uganda, but attains its maximum development in the eastward portion of the Ivory Coast Colony.

Dr. Harms (4) originally divided this genus into 3 sections—namely, *Euentandrophragma, Choriandra* and *Pseudo-entandrophragma*—adding later a fourth section, *Neo-entandrophragma*. It is in the first section, *Eu-entandrophragma*, that Harms departs most radically from the old nomenclature. On the West Coast he recognizes but a single species, *E. angolense* C. DC., reducing the other species to the rank of varieties. Of these, var. *macrophyllum* (Chev.), var. *dolichocarpum* (Harms), var. *beninense* (Harms), and var. *septentrionale* (Chev.) are covered in this paper.

In the above connection, it is timely to state that the present study substantiates in every way the four sub-genera recognized by Harms; critical examination of the West Coast *Entandrophragma* woods has served to indicate that they fall naturally into four groups, each easily distinguished by specific gross and minute characteristics.

General description of the wood.

As previously pointed out, four groups of *Endandrophragma* woods can be recognized on the basis of structure, these four groups corresponding with the Sections (Sub-genera) created by Harms and bearing out his contention that this is a logical division of the genus. In fact, so wide are the anatomical departures in the woods of these four sections that it has seemed unadvisable to attempt to prepare a general description which would hold for the woods of this genus but rather, in lieu of this, to insert a key in the text dealing with the salient anatomical features of the woods of the respective sections.

Key to the woods of Entandrophragma, arranged according to Sections (Sub-genera)

1. Heartwood reddish brown, often with a purplish cast, medium hard and heavy; pores 3–15 per mm.²; paratracheal- and metatracheal-zonate parenchyma in wavy, more or less broken and irregularly spaced lines, or wanting; terminal parenchyma in straightish lines; fibers thin-walled, septate or non-septate, with or without gummy infiltration; rays 1–7 (mostly 3–4) seriate (3–5 seriate in *E. angolense*, var. *macrophyllum*), storied or unstoried; crystals sometimes present in the "upright" cells 2

1. Heartwood dark red-brown to deep wine-red, hard and heavy; pores 0–6 per mm.²; zonate parenchyma in straightish or wavy, continuous and regularly spaced bands; fibers thin- or thick-walled, non-septate; rays 1–6 (mostly 3–4) seriate, unstoried, homogeneous or heterogeneous; crystals wanting in the "upright" cells.

                                        Sec. *Choriandra*
                                        E. candollei

    2. Sheaths of paratracheal parenchyma 1–4 seriate; paratracheal- and metatracheal-zonate parenchyma wanting or relatively sparse; infiltration copious in the fibers; rays heterogeneous, generally unstoried; crystals very rare in the "upright" cells ....Sec. *Eu-entandrophragma*
                                   E. angolense and its varieties.

    2. Sheaths of paratracheal parenchyma 1–2 seriate; paratracheal- and metatracheal-zonate parenchyma abundant, in wavy, continuous, or broken lines; infiltration sparse in the fibers; rays homogeneous and heterogeneous, storied or unstoried; crystals present or wanting in the "upright" cells 3

3. Wood with distinct cedary odor; rays mostly 3–4 seriate, generally characteristically storied; crystals frequent in the "upright" cells ....Sec. *Pseudo-entandrophragma*
                                       E. cylindricum

3. Wood odorless; rays mostly 2–3 seriate, unstoried or rarely storied in restricted areas; crystals wanting in the "upright" cells ....Sec. *Neo-entandrophragma*
                                       E. utile

SECTION CHORIANDRA

Entandrophragma candollei Harms

General characteristics of the wood:

Sapwood wide, grayish-white to light reddish-brown, grading gradually into the heartwood; heartwood dark red-brown to deep wine-red (darker than in the other species of Entandrophragma); lustrous to dull; odorless; somewhat rough to the feel; medium hard to hard; medium heavy (Sp. Gr. 0.55–0.67); medium fine to somewhat coarse textured; straight or interlocked grained, and in the latter instance showing a ribbon figure on the quarter. Growth rings fairly distinct, delineated by a line of terminal parenchyma, and occasionally in addition by a narrow band of denser fibrous tissue at the outer margin.

Pores scarce to fairly numerous, uniformly distributed, solitary or in short radial groups, medium large to large and distinctly visible with the naked eye, often occluded with dark red infiltration or occasionally with white deposits; vessel lines distinct or somewhat obscure, depending on the color of the wood. Parenchyma in numerous whitish or reddish, wavy or nearly straight, continuous or broken lines which may or may not be visible with the naked eye. Rays fairly numerous, of nearly uniform width, visible with the naked eye in light-colored samples of the wood, indistinct without a hand lens in dark-colored stock; ray fleck low to medium high, obscure to fairly conspicuous, usually darker than the background. Ripple marks absent, or very irregular and then evident only in small areas, about 15–20 per cm.

Minute anatomy:

Growth rings delineated by a 4–5 seriate band of terminal parenchyma preceded occasionally by a denser zone of several rows of radially flattened fibers.

Vessels generally solitary or in radial groups of 2–3, occasionally in longer radial rows (up to 7), rarely in tangential groups of 2–3 or in small nests, encircled by a 1–3 seriate sheath of parenchyma frequently interrupted by fibers or rays contiguous to the vessel, often connected by a 4–10 seriate band of zonate parenchyma, 0–6 (av. 3) per mm.^{2}; orifices round or oval, the largest 250–300 (av. 180–250) microns in diameter; segments 150–500 microns long, tailed or truncate; lateral walls 6–12 microns thick; perforations simple, round or oval, horizontal or slightly oblique; inter-vessel pits numerous, crowded, minute, rounded or irregularly polygonal, 2–3 microns in diameter, the apertures of 2-many often confluent; pits leading to rays numerous to each cell, similar to the inter-vessel pits; tyloses absent; dark-red infiltration and chalky inclusions abundant.

Fibers fine to medium fine, thin- to thick-walled, inconspicuously arranged in radial rows, in 15–50 seriate concentric bands which alternate with bands of zonate parenchyma, non-gelatinous or gelatinous (the latter in layers or intermingled with fibers of the normal type), non-septate or rarely septate, rounded or angular and then frequently rectangular in the transverse section, 16–32 microns in diameter, 700–2100 microns long; walls of non-gelatinous fibers 3–6 microns thick, the gelatinous layer when present, 6–8 microns thick; inter-fiber pits more abundant on the radial walls, minute, 1–3 microns in diameter, rounded, bordered, with long slit-like apertures.

Parenchyma (a) paratracheal, (b) paratracheal-zonate. (c) metatracheal, and (d) terminal: (a) *paratracheal* parenchyma in a 1–3 seriate sheath which is often interrupted by fibers and rays contiguous to the vessel; cells thin-walled, variable in shape and size, usually flattened to conform to the vessel wall, with maximum diameter of 20–60 microns, 60–140 microns in length; dark red infiltration abundant; crystals wanting; starch grains not observed; (b) *paratracheal-zonate* parenchyma in 5–10 seriate, somewhat wavy, concentric bands which alternate with bands of fibrous tissue; (c) *metatracheal* parenchyma scarce, the cells solitary or in small groups of 2–4; (d) *terminal* parenchyma in a 2–4 plus seriate, straightish line; cells of " b," " c," and " d " parenchyma thin-walled, the diameter about twice that of the fibers, rounded or angular and then frequently rectangular in the transverse section, 24–50 microns wide, 50–100 microns long; reddish-brown infiltration abundant; crystals and starch grains not observed.

Rays 4–7 per mm., uniform in width, 1–6 (mostly 3–5) seriate, unstoried or rarely storied (in the latter instance arranged in somewhat irregular, diagonal rows of sporadic occurrence), heterogeneous or rarely homogeneous, up to 100 microns in width, and 35 cells and 900 microns (av. 10–25 cells and 300–600 microns) in height; "upright" cells solitary at the ends of the ray (*t*), 40–80 microns high, 24–30 microns wide, 50–80 microns long; "horizontal" cells uniform in shape and size, rounded or slightly oblong (*t*), 20–40 microns high, 12–24 microns wide, 80–240 microns long; dark red infiltration abundant; crystals and starch grains not observed.

Material: Krukoff's Nos. 10 from Gold Coast; 100 from Ivory Coast; 142, 168, 170, 172 from Cameroon; 186 from Nigeria, all backed by herbarium material.

SECTION EU-ENTANDROPHRAGMA

Entandrophragma angolense C. DC.

As previously pointed out, this species, placed by Harms in the Section *Eu-entandro-phragma,* includes a number of varieties which some taxonomists consider as distinct species. Since minor, but apparently quite constant anatomical variations have been found in the woods of these, descriptions of four of these forms—namely, *E. angolense* C. DC., var. *macrophyllum* (Chev.), *E. angolense* C. DC., var. *beninense* Harms, *E. angolense* C. DC., var. *septentrionale* (Chev.), and *E. angolense* C. DC., var. *dolicho-carpum* Harms—are included in this report.

General characteristics of the wood:

Sapwood wide, purplish-gray to pale rosy-red; heartwood light red to reddish-brown, usually with a distinct purplish cast; lustrous; odorless; with smooth feel; medium hard; light to medium heavy (Sp. Gr. 0.49–0.62); medium fine textured; straight or interlocked grained. Growth rings usually distinct, delineated by a line of terminal parenchyma and in addition frequently by a narrow zone of denser fibrous tissue. Pores scarce to fairly numerous, uniformly distributed (Specimen No. 23, from a broken top, showed a ring porous arrangement of vessels), medium large (distinctly visible with the naked eye), surrounded by a halo of parenchyma (10 X), open or occluded with lustrous light to dark red infiltration; vessel lines distinct, appearing as grooves of a somewhat darker color than the background. Terminal parenchyma in quite closely spaced concentric lines which are often obscure to the naked eye; paratracheal parenchyma forming a halo about the pores, distinct with a 10 X hand lens. Rays fairly numerous, of nearly uniform width, scarcely visible or indistinct with the naked eye; ray fleck low to medium high, distinct. Ripple marks absent.

Minute anatomy:

Growth rings delineated by a 1–3 seriate line of terminal parenchyma, and occasionally in addition by a band consisting of several rows of thicker walled, radially flattened fibers.

Vessels mostly solitary or in radial groups of 2–3, rarely in tangential groups of 2–3 or in small nests, encircled by a 1–4 (mostly 2–3) seriate sheath of parenchyma which is occasionally interrupted by rays, 4–8 (av. 6) per mm.^{2}; orifices round or oval, the largest 180–220 (av. 140–180) microns in diameter; vessel segments 150–500 microns long, tailed on one or both ends or truncate; lateral walls 4–10 microns thick; perforations simple, round or oval, horizontal or slightly oblique; inter-vessel pits numerous, crowded, minute, rounded or irregularly polygonal, 2–3 microns in diameter, the apertures of 2-many often confluent; pits leading to rays numerous to each cell, similar to the inter-vessel pits, 2–4 microns in diameter; dark red and white infiltration sparse to copious; tyloses absent.

Fibers fine to medium fine, thin-walled, inconspicuously arranged in radial rows, non-septate, non-gelatinous, frequently plugged with dark red infiltration, rounded or angular and then frequently rectangular in the cross section, 16–30 microns in diameter, 900–2000 microns long; walls 2–4 microns thick; inter-fiber pits minute, rounded, simple or nearly so.

Parenchyma (*a*) paratracheal, (*b*) metatracheal, and (*c*) terminal, in cambiform rows along the grain which are often further subdivided into loculi containing dark red deposits: (*a*) *paratracheal* parenchyma (1) in a 1–4 (mostly 2–3) seriate sheath which is occasionally interrupted by rays contiguous to the vessel, (2) often extending from the flanks of the vessel as short wing-like projections and forming an eyelet with the orifices, (3) rarely uniting 2-several vessels, forming interrupted, wavy 2–6 seriate bands; cells thin-walled, those contiguous to the vessels appreciably flattened, 20–60

microns wide and 20–180 microns long, the others somewhat wider than the largest fibers, mostly rectangular in the transverse section, 25–35 microns wide, and 60–160 microns long; reddish-brown infiltration frequent; crystals not observed; starch grains few to very abundant; (*b*) *metatracheal* parenchyma sparse, the cells isolated or in small groups, similar to those of the paratracheal-zonate parenchyma; (*c*) *terminal parenchyma* in 2–5 seriate, straightish, more or less obscure lines; cells comparable to those of the paratracheal-zonate parenchyma.

Rays 3–6 per mm., 2–7 (mostly 3–5) seriate, unstoried, heterogeneous, up to 100 microns in width, 45 plus cells and 1000 plus microns (av. 15–30 cells and 400–700 microns) in height; "upright" cells usually restricted to one cell at each end of the ray (*t*), 40–80 microns high, 20–30 microns wide, 40–80 microns long; "horizontal" cells rounded or oblong (*t*), 20–30 microns high, 12–20 microns wide, 80–240 microns long; pits leading to vessels numerous to each ray cell, similar to the inter-fiber pits, 2–4 microns in diameter; red-brown infiltration abundant; crystals and starch grains not observed.

Material: Krukoff's Nos. 23 and 31 from the Gold Coast, 59 and 76 from the Ivory Coast, all with herbarium material; Vigne's No. 1496 from Gold Coast, herbarium material determined, at Oxford as *E. macrophyllum* Chev.

Entandrophragma angolense C. DC., var. beninense Harms

General characteristics of the wood:

Sapwood light rosy-red; heartwood reddish-brown with a purplish cast; lustrous to somewhat dull; odorless; medium hard; medium heavy (Sp. Gr. approx. 0.6); fine textured and interlocked grained. Growth rings distinct, delineated by a fine line of terminal parenchyma. Pores fairly numerous, evenly distributed, small to fairly large (barely visible with the naked eye), open or plugged with dark red infiltration, surrounded by a halo of paratracheal parenchyma (10 ×). Terminal parenchyma barely visible with the naked eye, in fine concentric lines which are somewhat lighter than the background; paratracheal parenchyma forming a faint halo about the pores and extending laterally from their flanks in very short tangential lines. Rays fairly numerous, barely visible with the naked eye; ray fleck medium high, darker than the background, distinct. Ripple marks absent.

Minute anatomy:

Similar to that of *E. angolense* var. *macrophyllum* with the following exceptions: *vessels* mostly solitary, rarely in radial groups of 2, surrounded by a 2–4 seriate sheath of parenchyma which often forms a definite eyelet with the orifice, 2–several often united tangentially by short, 2–5 seriate extensions of paratracheal-zonate parenchyma, 7–10 (av. 8) per mm.², orifices round or polygonal, the largest 100–120 microns in diameter; *fibers* fine, septate or non-septate, 20–24 microns in diameter, 1200–2000 microns long; *rays* 4–7 per mm., 2–5 (mostly 3–4) seriate, up to 90 microns in width, 20 cells and 500 microns (av. 8–12 cells and 300–400 microns) in height.

Material: Krukoff No. 180 from Nigeria, backed by herbarium material.

Entandrophragma angolense C. DC. var. dolichocarpum Harms

General characteristics of the wood:

Sapwood narrow, very light reddish-brown, grading gradually into the heartwood; heartwood reddish-brown with a grayish-purple cast; lustrous; odorless; medium hard; light to medium heavy (Sp. Gr. approx. 0.55); medium fine textured; cross grained. Growth rings distinct, delineated by an even whitish line of terminal parenchyma, plainly visible with the naked eye. Otherwise similar to *E. angolense* var. *macrophyllum.*

Minute anatomy:

Similar to that of *E angolense* var. *macrophyllum* with the following exceptions: *fibers* fine, non-septate or rarely septate, 16–28 microns in diameter, 1000–2000 microns long; *rays* 3–4 per mm., 1–5 (mostly 3–5) seriate, up to 60 microns in width, 25 cells and 700 microns (av. 15–20 cells and 500–600 microns) in height.

Material: Krukoff, No. 111 from French Gabon, backed by herbarium material.

Entandrophragma angolense C. DC. var. *septentrionale* (Chev.)

General characteristics of the wood:

Sapwood rosy-red to light reddish-brown, grading very gradually into the heartwood; heartwood reddish-brown, often with a purplish cast and golden luster; odorless; medium hard; light to medium heavy (Sp. Gr. approx. 0.6); medium fine textured; unusually cross grained and often exhibiting distinct ribbon and curly figures on the quarter, hence the trade name, Acajou frisé. Growth rings distinct, delineated by an even line of terminal parenchyma. Otherwise similar to *E. angolense* var. *macrophyllum.*

Minute anatomy:

Similar to that of *E. angolense* var. *macrophyllum* with the following exceptions: *rays* 3–5 per mm., 2–5 (mostly 4) seriate, up to 80 microns in width, 35 cells and 1000 microns (av. 15–22 cells and 600–700 microns) in height.

Material: Krukoff's No. 178 from Nigeria, backed by herbarium material.

SECTION PSEUDO-ENTANDROPHRAGMA

Entandrophragma cylindricum Spr.

General characteristics of the wood:

According to French authorities, two distinct varieties of Aboudikro wood occur on the Ivory Coast, (1) a light rosy-red sort, and (2) a dark red-brown type; both darken on exposure, but the first remains the lighter. The wood of *E. cylindricum* from Cameroon and Nigeria is generally of a darker hue than that from the Gold and Ivory Coast Colonies. Sapwood white to light rosy-red, 2–6 cm. thick, grading gradually into the heartwood; heartwood light rosy-red to red-brown with a purplish cast; often with a golden luster; with a distinct cedary odor; medium hard to hard; medium heavy to heavy (Sp. Gr. 0.6–0.85); medium fine textured; straight to interlocked grained and then exhibiting rosy, ribbon, or mottled figures in quarter sawn stock; works well under tools and finishes quite smoothly. Growth rings distinct, delineated by a line of terminal parenchyma preceded by a narrow band of somewhat denser fibrous tissue. Pores fairly numerous, uniformly distributed, solitary or in short radial rows, and in addition occasionally more or less obscurely grouped in somewhat wavy, tangential lines, medium large and distinct with the naked eye, open or plugged with dark red infiltration or with white deposits; vessel lines distinct, frequently occluded with infiltration. Terminal parenchyma distinctly visible with the naked eye, appearing as straightish, white lines; paratracheal- and metatracheal zonate parenchyma in wavy interrupted or continuous bands which are more or less indistinct with the naked eye. Gum canals, when present, appearing in the transverse section as concentric lines which are plainly visible without magnification, and as streaks along the grain in the radial section. Rays fairly numerous, uniform in width, barely visible or indistinct with the naked eye; ray fleck low but fairly conspicuous, usually darker than the background. Ripple marks generally present, very regular, distinct with the naked eye, about 20–25 per cm.

Minute anatomy:

   Growth rings delimited by a 2–7 seriate band of terminal parenchyma.

   Vessels evenly distributed, solitary or in radial rows of 2–3, occasionally in longer radial rows (up to 7), rarely in tangential groups of 2–3 or in small nests, encircled by a 1–2 seriate sheath of parenchyma which is frequently interrupted by fibers and rays contiguous to the vessel, often joined tangentially by 2–10 plus seriate bands of zonate parenchyma, 4–15 (av. 7) per mm.[2]; orifices round or oval, the largest 180–250 microns (av. 140–160 microns) in diameter; vessel segments 150–500 microns long, truncate or rarely tailed; lateral walls 6–12 microns thick; perforations simple, round or oval, horizontal or nearly so; inter-vessel pits very numerous, crowded, minute, round or irregularly polygonal, 1–3 microns in diameter, the apertures of 2-many often confluent; pits leading to contiguous rays numerous to each cell, similar to the inter-vessel pit; tyloses absent; reddish-black and white infiltration abundant.

   Fibers medium fine, thin- to moderately thick-walled, inconspicuously arranged in radial rows, septate or non-septate, non-gelatinous, rounded or angular and then frequently rectangular in the transverse section, 20–30 microns in diameter, 700–1700 microns long; walls 3–6 microns thick; inter-fiber pits small, round, simple or bordered; dark red infiltration occasional.

   Parenchyma (a) paratracheal, (b) paratracheal-zonate, (c) metatracheal, and (d) terminal, mostly in cambiform rows along the grain the units of which are often further subdivided into loculi containing solitary crystals: (a) *paratracheal* parenchyma forming a 1–2 seriate sheath which is often interrupted by rays and fibers contiguous to the vessel; cells thin-walled, usually flattened to conform to the vessel wall, with maximum diameter of 30–70 microns, 40–100 microns long; dark reddish infiltration frequent; solitary crystals occasional; starch grains scarce to abundant; (b) *paratracheal-zonate* parenchyma in wavy, 2–6 seriate interrupted or continuous bands which, together with the metatracheal and terminal parenchyma, occasionally form an irregular reticulum with the rays, these tracts alternating with others containing little or no zonate parenchyma; (c) *metatracheal* parenchyma (1) occurring in part as solitary cells or in small groups (2) for the most part zonate, in 2–6 seriate, interrupted or continuous, wavy bands; (d) *terminal* parenchyma in straightish, occasionally branching, 2–10 plus seriate bands; cells of "b," "c," and "d" types of parenchyma rounded or rectangular in the transverse section, about twice as wide as the fibers, 28–50 microns wide, 50–120 microns in length; reddish-brown infiltration scarce to abundant; crystals frequent, confined to the loculated cells; starch grains scarce to abundant.

   Gum canals, when present, arranged in concentric rows (x), imbedded in terminal parenchyma.

   Rays 4–6 per mm., uniform in width, 2–5 (mostly 3–4) seriate, usually regularly storied, heterogeneous or occasionally homogeneous, up to 80 microns wide, mostly 15–20 cells and 300–400 microns (rarely 30 plus cells and 1300 plus microns) in height; "upright" cells restricted to one at each end of the ray (t), 40–50 microns high, 24–30 microns wide, 50–60 microns long; "horizontal" cells very uniform in size and shape, rounded or oblong (t), 20–30 microns high, 12–20 microns wide, 80–180 microns long; pits leading to vessels numerous to each cell, similar to those of the inter-vessel type, 2–4 microns in diameter, with distinct border; globules of reddish-brown infiltration abundant; crystals frequent in the "upright" cells, solitary; starch grains absent or very sparse.

   Ripple marks present, traceable to storied rays.

   Remarks: Occasionally confused with the wood of *Guarea cedrata* (Chev.) Pell. but generally darker in color, ranging from rosy-red to reddish-brown with a purplish cast, and usually with well defined ripple marks. *Guarea cedrata*, in comparison, is light rosy-red, and lacks ripple marks. The following microscopical characters will serve to distinguish these woods:

Vessels mostly solitary or in radial groups of 2, the largest 180–250 microns in
    diameter; rays 2–5 (mostly 3–4) seriate, generally storied; "upright" cells,
    crystalliferous ....*E. cylindricum*
Vessels solitary or in groups of 2–6, the largest 160–180 microns in diameter;
    rays 1–3 (mostly 1–2) seriate, unstoried; "upright" cells, non-crystalliferous.
                                                                                G. cedrata

Material: Krukoff's Nos. 1, 11, 19, 40 from the Gold Coast; 73 and 74 from the
Ivory Coast; 150, 151, 156, 162, 163 and 174 from Cameroons; 177 from Nigeria, all
backed by herbarium material. Kennedy's No. 315 from Nigeria herbarium material at
Oxford; Vigne's No. 1825 from the Gold Coast, herbarium material at Syracuse.

SECTION NEO-ENTANDROPHRAGMA

Entandrophragma utile Spr.

General characteristics of the wood:

Sapwood grayish-white to pale rosy-red, 1–6 cm. thick, grading gradually into the
heartwood; heartwood light reddish-brown to purplish brown, often with a distinct
golden luster; odorless; smooth to the feel; medium hard; soft to medium heavy (Sp.
Gr. 0.54–0.65); fine textured; straight or interlocked grained and then often exhibiting
rosy or ribbon figures on the quarter; works easily under tools and polishes fairly well.
Growth rings distinct, delineated by a fine, straightish line of terminal parenchyma.
Pores scarce to fairly numerous, evenly distributed, solitary or in short radial groups,
medium large (distinctly visible to the naked eye), open or plugged with dark red
infiltration or rarely with whitish deposits; vessel lines distinct, occluded with dark
red, or rarely with white, deposits. Terminal parenchyma in fine, straightish, white or
reddish, concentric lines; paratracheal- and metatracheal-zonate parenchyma scarce to
abundant, in broken, wavy, tangential lines which are inconspicuous with the naked eye.
Rays fairly numerous, uniform, barely visible with the naked eye; ray fleck low to
medium high, conspicuous, darker than the background. Ripple marks present or want-
ing, when present, never very regular or distinct and generally of local occurrence,
15–25 per cm.

Minute anatomy:

Similar to that of *E. cylindricum,* with the following exceptions; *growth rings*
present or wanting, when present, delineated by a 2–4 seriate band of terminal paren-
chyma; *paratracheal* parenchyma sparse to fairly abundant, forming a 1 (rarely 2)
seriate sheath; *rays* 5–7 per mm., 1–4 (mostly 2–3) seriate, storied or unstoried, when
storied, arranged more or less irregularly in somewhat indistinct diagonal rows which
are usually of local occurrence, both heterogeneous and homogeneous, up to 20 microns
wide, occasionally 70 plus cells (av. 15–40 cells) and up to 1700 microns (av. 350–800
microns) in height; reddish-brown infiltration sparse to abundant; crystals not observed.

Material: Krukoff's Nos. 2 and 7 from the Gold Coast; 101 from the Ivory Coast;
139 from French Gabon; 148, 152 and 173 from Cameroon, all backed by herbarium
material.

Lovoa Harms

Botanical description.

Large trees 70–90 ft., occasionally up to 110 ft. in height, and 3–4 ft. in
diameter, with a straight, cylindrical, short buttressed trunk free of branches
for 50–60 ft. supporting a dense, well developed crown. Leaves large, pin-
nately compound with 3–6 pairs of sub-opposite leaflets. Flowers numerous,
small, in large, lax, glabrous, sub-corymbose panicles. Fruit a very charac-

teristic, prismatic capsule approximately 1 cm. in diameter which dehisces by four very thin, membranous valves; seeds with a terminal wing pointing toward the apex of the columella (the reverse holds in *Entandrophragma* and *Pseudocedrela*).

Size and distribution.

Nine species of Lovoa have been described, all restricted to tropical Africa, but of these only one, *L. klaineana* Pierre, is well known to science. A tolerant tree restricted to West Africa and extending from Sierra Leone to Gabon. Common on the Ivory Coast and in the Western Province of the Gold Coast, likewise in southern Nigeria, the Cameroons and Gabon. Occurring single or in groups in closed forest of the moist type and exhibiting a preference for rich clay soils with ample moisture.

General description of the wood.

Since *L. klaineana* is the only species of commercial importance at the present time and material of the other species was not available for study, information under this heading is omitted. See under " Species " for data on this wood.

Lovoa klaineana Pierre

General characteristics of the wood:

Sapwood light grayish-brown; heartwood grayish-brown to dark chocolate-brown, with a distinct golden lustre; odorless; smooth to the feel; medium hard; light to medium heavy (Sp. Gr. 0.5–0.62); fairly fine textured; interlocked grained, the quarter sawn lumber showing a very pronounced ribbon figure consisting of alternating golden-brown and somewhat dull chocolate-brown bands (this fact accounts for the trade name of Black Mahogany which is occasionally applied to this wood); working easily under tools and finishing smooth. Growth rings indistinct or faintly marked by a narrow zone of dense fibrous tissue at the outer margin. Pores fairly numerous to numerous, evenly distributed, solitary or in short radial groups, medium large and distinctly visible with the naked eye, open or occluded with blackish gummy infiltration. Zonate parenchyma absent; paratracheal parenchyma indistinct. Black concentric lines consisting of gum canals occluded with blackish gummy infiltration sometimes present, sporadic to fairly numerous, conspicuous on both the transverse and radial surfaces. Rays fine, numerous, evenly spaced, just visible with the naked eye; ray fleck medium high, somewhat darker than the background and hence fairly conspicuous. Ripple marks absent.

Minute anatomy:

Growth rings indistinct, even at high magnifications.

Vessels solitary and in short radial groups of 2–6, more rarely in tangential groups of 2–3 or in small nests, usually encircled by a 1–3 seriate sheath of paratracheal parenchyma which often extends laterally and forms eyelets with the orifices, 4–19 (av. 10) per mm.^{2}; orifices rounded or rarely oval, the largest 220–250 (av. 140–200 microns) in diameter; segments 150–600 microns long, tailed or truncate; lateral walls 4–10 microns thick; perforations simple, rounded or oval, horizontal or slightly oblique; inter-vessel pits numerous, crowded, small, rounded, 3–4 microns in diameter, the apertures of many often confluent; pits leading to contiguous rays similar to the inter-vessel pits, numerous to each cell, with distinct borders; tyloses absent; dark brown to black infiltration copious.

Fibers fine to medium fine, thin-walled, inconspicuously arranged in radial rows, non-septate, non-gelatinous, rounded or somewhat angular in the cross section, 16–30 microns

in diameter, 500–1800 microns long; walls 2–3 microns thick; inter-fiber pits confined to the radial walls, fairly numerous, small, rounded, 2–4 microns in diameter, bordered, with very narrow, slit-like apertures; infiltration not observed.

Parenchyma (*a*) paratracheal, (*b*) metatracheal, and (*c*) metatracheal-zonate, in cambiform rows of 2–8 cells along the grain which are often further subdivided into strings of 2-many loculi, each with a large, solitary crystal: (*a*) *paratracheal* parenchyma (1) in a 1–3 seriate sheath which is frequently interrupted by fibers or rays contiguous to the vessel and is often prolonged laterally, forming an eyelet with the orifice, (2) rarely extending in 1–3 seriate bands across the rays and uniting several vessels; (*b*) *metatracheal* parenchyma sparse to abundant, the cells solitary or in small groups; cells of both " a " and " b " parenchyma somewhat larger than the largest fibers, thin-walled, very variable in shape (*x*), 20–40 microns wide, 40–250 microns long; dark-colored infiltration frequent; catenate strings of crystal-bearing loculi numerous; starch grains rare; (*c*) *metatracheal-zonate* parenchyma very rare, when present, grouped in many seriate concentric bands which are *always* associated with gum canals; cells somewhat wider than the largest fibers, thin-walled, mostly rectangular in cross section, 20–35 microns in diameter, 20–60 microns long; dark brown infiltration copious; crystals not observed; starch grains rare.

Rays 3–5 per mm., 2–5 (mostly 4) seriate, unstoried, homogeneous or rarely hetero-geneous, up to 60 microns in width and 45 cells and 850 microns (av. 15–30 cells and 300–600 microns) in height: " upright " cells, when present, solitary at the ends of the ray (*t*), 30–60 microns high, 16–24 microns wide, 40–60 microns long; " horizontal " cells oval (*t*), 16–30 microns high, 12–24 microns wide, 40–100 microns long; pits leading to contiguous vessels numerous to each cell, rounded, 3–4 microns in diameter, with distinct borders; dark brown to black infiltration copious; crystals not observed.

Material: Krukoff's Nos. 15, 16, 17 and 18 from the Gold Coast; 68 and 69 from the Ivory Coast; 160 from French Cameroon, all backed by herbarium material; Kennedy's No. 319 with herbarium material at Oxford; Thompson, Moir and Galloway C. 1.; Baumgartner B. 3, Comité National des Bois Coloniaux, Paris, Syracuse No. D–10; Agence Général des Colonies, Paris, No. E–11.

Khaya A. Juss.

Botanical description.

Medium sized to very large trees, frequently over 120 ft. in height and 3–5 ft., rarely up to 10 ft., in diameter, with a straight cylindrical trunk clear of branches for upward of 70–100 ft. (except *K. senegalensis* A. Juss.), and a large buttress which often reaches upwards to 20 ft. above the ground. Leaves alternate, abruptly pinnate, with 2–7 pairs of opposite or sub-opposite leaflets. Flowers 4–5-merous, in axillary panicles. Fruit a 4–5 celled. 4–5 valved (rarely 3-valved, in *K. anthotheca* C. DC., Kr. No. 171), more or less globose, woody capsule; seeds numerous in each cavity, winged all around.

Size and distribution.

This genus is confined to the tropical and sub-tropical regions of Africa and Madagascar, the center of distribution occurring on the West Coast of the continent. Fully 15 species have been described, some of which will undoubtedly be dropped, as the tropical flora of Africa becomes better known.

Seven species of *Khaya* are described in this report—namely, *K. grandifoliola* C. DC., *K. ivorensis* A. Chev., *K. klainei* Pierre, *K. senegalensis* A. Juss., *K. anthotheca* C. DC., *K. euryphylla* Harms, and *K. madagascarensis* Jum. et Pierre; six of these occur on the West Coast, the seventh in Mada-

gascar. The scientific names employed are those affixed to the herbarium sheets by Dr. H. Harms; it should be noted in this connection that *K. klainei* Pierre, which is regarded by the British scientists as a synonym of *K. ivorensis* A. Chev., and *K. euryphylla* Harms, which also has been considered by them as synonymous with *K. anthotheca* C. DC., are here retained as separate species.

K. ivorensis has a wide range, extending from the Ivory Coast to Gabon; it attains its maximum frequency and development in the Ivory Coast and Gold Coast Colonies. *K. grandifoliola* extends through French Guinea to the Cameroons and thence eastward to Ubanchi-Shari-Chad and to the Anglo-Egyptian Sudan; it is a transition-forest tree and is found between the closed forests of the dry type and savannah forest. *K. klainei* is known to occur in Gabon and is fairly common in the closed forest of the moist type in the region of Lake Anenghé; according to Krukoff, this species often grows side by side with *K. ivorensis*. *K. senegalensis* is also widely distributed, ranging from the French Sudan to the Cameroons, and thence eastward to the Anglo-Egyptian Sudan and Uganda; it is confined to savannah forest and is frequently gregarious in small groups. *K. anthotheca* is found in the Gold Coast Colony and in the Cameroons, while *K. euryphylla* is definitely known to occur in the Ivory Coast and in the Cameroons, but its range is undoubtedly more extensive, requiring further investigation to ascertain its distribution in some of the British Colonies. This last species, either alone or in admixture with *K. anthotheca*, also occurs in the Gold Coast and in Nigeria (at least on the banks of the Ovia river in the Ifon district of Benin, in the province of Ondo and at Lokoja, and in Angola in the mountain forests of Golungo Alto and Dembos). Finally, *K. madagascarensis* has been reported from the forests of le Boina and L.' Ambongo, in Madagascar.

General description of the wood.

The woods of *K. ivorensis, K. klainei, K. grandifoliola, K. senegalensis,* and *K. madagascarensis* range from reddish-brown to dark red-brown and often have a purplish cast. Those of *K. euryphylla* and *K. anthotheca*, in contrast, are decidedly lighter in color, varying from pale rosy-red to light reddish-brown. All of these timbers are extremely close anatomically, so close in fact that they cannot be separated with any degree of certainty.

Khaya ivorensis A. Chev.

General characteristics of the wood:

Sapwood grayish-white to light pinkish-red, 1-6 cm. wide, grading gradually into the heartwood; heartwood pale rosy-red to dark reddish-brown, often with a purplish cast and usually with a distinct golden lustre; odorless; soft to moderately hard; light to moderately heavy (Sp. Gr. 0.46-0.67); medium fine textured; straight to interlocked grained, in the latter instance often showing on the quarter a variety of figures such as "ribbon," "striped and roey," or more rarely, "mottled," "rain drop" or "blister"; works easily and finishes smooth, taking a very good polish. Growth rings fairly distinct (especially in stock from deciduous forests), delineated by a darker zone of fibrous

tissue at the outer margin and occasionally, in addition, by a line of terminal parenchyma. Pores scarce to fairly abundant, evenly distributed or frequently less numerous or wanting in the outer portion of the ring, solitary or in short radial groups, medium large (visible to the naked eye), often plugged with dark reddish-brown or rarely with white infiltration; vessel lines distinct, occluded with reddish-brown or white infiltration. Terminal parenchyma absent, or sporadic and inconspicuous; paratracheal parenchyma indistinct (even with a 10 X hand lens). Gum canals sometimes present, arranged in well defined concentric lines which are visible with the naked eye in the transverse section. Rays of two kinds, the larger distinctly visible with the naked eye, the smaller barely visible with a 10 X hand lens; ray fleck medium high, conspicuous against the lighter background. Ripple marks absent or sporadic, irregular and confined to restricted areas.

Minute anatomy:

Growth rings frequently obscure at higher magnifications, delineated by several rows of radially flattened fibers and a zone devoid of vessels, and rarely, in addition, by a 2–4 seriate, poorly defined band of terminal parenchyma.

Vessels solitary and in short radial groups of 2–8, occasionally in tangential rows of 2–5 or in small nests, usually encircled by a 1–3 seriate sheath of parenchyma which is often interrupted by fibers or rays contiguous to the vessel, 1–12 (av. 6) per mm.^{2}; orifices round or slightly oval, the largest 180–240 (av. 140–180) microns in diameter; segments 200–500 microns long, tailed or truncate; lateral walls 6–12 microns thick; perforations simple, round, horizontal or nearly so; inter-vessel pits very numerous, crowded, minute, round or irregularly polygonal, 2–4 microns in diameter, the apertures of 2-many often confluent; pits leading to contiguous rays numerous to each cell, similar to the inter-vessel pits, round, 3–4 microns in diameter, with distinct borders; tyloses absent; dark reddish-brown to black infiltration and white deposits abundant.

Fibers thin- to thick-walled, arranged in somewhat indistinct radial rows, septate or rarely non-septate, gelatinous and non-gelatinous in alternate bands or the two types intermingled, rounded or angular and then frequently rectangular in the cross section, 20–32 microns in diameter, 800–1800 microns long; walls of the non-gelatinous fibers 4–8 microns thick, the gelatinous layer, when present, 2–4 microns in thickness; inter-fiber pits small, simple or bordered, round, with narrow slit-like apertures; lumina occasionally plugged with dark red infiltration.

Parenchyma (*a*) paratracheal, (*b*) metatracheal, and (*c*) terminal, in cambiform rows of 2-many units along the grain which are occasionally divided further into loculi, each containing a large solitary crystal, or undivided and occluded with dark gummy infiltration: (*a*) *paratracheal* parenchyma sparse to abundant, forming a 1–3 seriate sheath which is often interrupted by fibers and rays contiguous to the vessel; cells thin-walled, usually flattened to conform to the vessel wall, with maximum diameter of 24–50 microns, 40–100 microns long; dark red to black gummy infiltration frequent; crystals present; starch grains absent or very scarce; (*b*) *metatracheal* parenchyma sparse, the cells solitary or in small groups, usually in the proximity of or flanking the rays; (*c*) *terminal* parenchyma never very prominent, sporadic and often absent in many consecutive growth rings, forming a 2–4 (rarely more) seriate line which is not sharply delineated from the fibrous tissue, not infrequently including 1-many rows of gum canals; cells of the "b" and "c" parenchyma thin-walled, about the width of the largest fibers, rounded or angular and then frequently rectangular in the transverse section, 16–36 microns wide, 60–120 microns long; dark red gummy infiltration occasional to very abundant; crystals rare; starch deposits absent or very sparse.

Rays 4–7 per mm., unstoried or obscurely storied and the storied rays then confined to restricted areas, of two sizes: (*a*) *large rays* 3–10 (mostly 4–7) seriate, up to 200 (av. 60–140) microns wide and 60 plus cells and 1500 plus microns (av. 15–40 cells and 500–1200 microns) in height, distinctly heterogeneous, the 1–5 "upright" cells at each

end of the ray (*t*) forming a long, attenuated tip, the cells on the flanks of the multi-seriate portion of the ray usually about twice as large as those of the core; (*b*) *small rays* 1–3 (mostly 1–2) seriate, 10–40 microns wide, 1–15 cells and 50–400 microns in height, frequently consisting entirely of "upright" cells; "upright" cells in the "a" and "b" rays 440–490 microns high, 30–40 microns wide, 40–60 microns long; "horizontal" cells squarish, round or oval (*t*), 10–30 microns high, 10–30 microns wide, 100–200 microns long; pits leading to contiguous vessels similar to the inter-vessel pits, numerous to each cell, 3–4 microns in diameter, round, with distinct border; reddish-brown infiltration abundant; rhomboid crystals present in the "upright" cells; starch deposits absent to scanty.

Material: Krukoff's Nos. 4, 5, and 6 from the Gold Coast deciduous forest; 9, 13, 14, 20, 24, 25, 26, 27, 28, 33, and 45 from the Gold Coast evergreen forest; 66, 70, and 75 from the Ivory Coast; 103 and 104 from French Gabon; 150 from Cameroon; 185 from Nigeria, all backed by herbarium material; Kennedy's No. 309 from Nigeria, herbarium material at Oxford; Vigne's No. 1501 from Gold Coast, herbarium material at Oxford; Mildbraed's No. 25, *K. ivorensis* var. *kamerunensis* Harms, herbarium material No. 5571 Mildbraed at Bot. Mus., Berlin; Trade Sample from Baumgartner, French Gabon, Syracuse No. B. 4; Muséum National d'Histoire Naturelle per Collardet, Syracuse Nos. D. 1, D. 2; Comité National de Bois Coloniaux, Paris, per Collardet, Syracuse Nos. D. 3—Acajou Bassam; D. 4—Figured African Mahogany; D. 5—Acajou rouge; Agence Général des Colonies, Paris, Syracuse No. E. 1.

Khaya grandifoliola C. DC.

General characteristics of the wood:

Sapwood light pinkish-brown, rather sharply delineated from the heartwood; heartwood reddish-brown, somewhat dull or with a distinct golden lustre; odorless; smooth to somewhat rough to the feel; medium hard to hard; medium heavy to heavy (Sp. Gr. approx. 0.70); medium fine textured; interlocked grained, and somewhat splintery and difficult to bring to a smooth surface. Growth rings distinct or wanting, when present, delineated by a fibrous zone or occasionally by a line of terminal parenchyma.

Minute anatomy:

Similar to that of *K. ivorensis*, with the following exceptions: *fibers* finer (14–22 microns in diameter) and generally thicker walled.

Material:

Vigne's No. 1803 from the Gold Coast, backed by herbarium material; No. 825 Oxford collection (*K. grandifolia* C. DC.); Syracuse No. 159–3052, from Nigeria.

Khaya klainei Pierre

General characteristics of the wood:

Sapwood grayish-white, 1–3 cm. wide, grading gradually into the heartwood; heartwood light rosy-red to very dark reddish- or occasionally chocolate-brown, usually with a pronounced golden lustre; odorless, moderately soft to hard; medium light (Sp. Gr. 0.52–0.60); fine to medium fine textured; interlocked grained and often with distinct ribbon figure on the quarter; working easily under tools and finishing smooth. Growth rings distinct, delineated by a zone of denser fibrous tissue and occasionally in addition by a faint line of terminal parenchyma, the lines, when present, either sporadic or abundant but never conspicuous to the naked eye.

Minute anatomy:

Similar to that of *K. ivorensis*, with the following exceptions: *vessels*, where radially grouped, in rows of 2–3 (generally 2–8 in *K. ivorensis*); sheath of paratracheal

parenchyma wider (1–4 seriate as compared to 1–3 seriate in *K. ivorensis*), occasionally forming short wings from the flanks of the vessels and extending laterally across adjacent rays; *metatracheal* parenchyma generally more abundant than in *K. ivorensis*; lines of *terminal* parenchyma frequently more numerous and regularly spaced than in the other *Khaya* species (including *K. ivorensis*).

Remarks: The wood of *K. klainei* is frequently attacked by the Gabon fly, the larvae of which mine through the trunk spirally, greatly reducing the commercial value of this fine timber.

Material: Krukoff's Nos. 106, 107, 114, 115, and 136 from French Gabon, all backed by herbarium material.

<center>*Khaya senegalensis* A. Juss.</center>

General characteristics of the wood:

Sapwood pink to light reddish-brown, 1–6 cm. thick; heartwood dark reddish-brown with a pronounced golden lustre; odorless, somewhat rough to the feel; medium hard and medium heavy (Sp. Gr. 0.70–0.75); medium fine textured; straight to interlocked grained and in the latter instance usually with a well defined ribbon figure on the quarter; working easily under tools and finishing fairly smoothly. Growth rings usually indistinct, rarely delineated by a faint line of terminal parenchyma. Pores fairly numerous, solitary or in short radial groups, evenly distributed or occasionally somewhat larger and more numerous in the inner portions and sparse or absent in the outer portions of growth rings, medium large to large and distinctly visible with the naked eye, frequently plugged with dark red to black infiltration and rarely with white deposits; vessel lines distinct, occluded with infiltration. Terminal parenchyma sporadic, often absent but in general more abundant and conspicuous than in *K. ivorensis*, in faint white lines; paratracheal parenchyma indistinct, with the naked eye, occasionally barely visible with a 10 × hand lens; ray fleck low to medium high, fairly conspicuous, usually darker than the background. Ripple marks present or absent, when present, restricted to small areas, about 15–20 per cm.

Minute anatomy:

Similar to that of *K. ivorensis*, with the following exceptions: *fibers* narrower (16–24 microns in diameter) and shorter (700–1400 microns), more rounded in the transverse section, always septate and non-gelatinous; sheath of *paratracheal parenchyma* occasionally extending in short, wing-like projections to adjacent rays; *metatracheal parenchyma* not infrequently abundant; large *rays* with 1–8 "upright cells" at each end (*t*).

Material: Vigne's No. 1559 from the Gold Coast, backed by herbarium material; Kersting's No. 1, herbarium material at Bot. Mus., Berlin; No. 1582 from Nigeria, Qxford collection; No. 866 from Nigeria, 1926.

<center>*Khaya madagascarensis* Jum. et Pier.</center>

General characteristics of the wood:

Heartwood very dark red-brown to purple, with distinct golden luster, medium hard, medium heavy. Vessels frequently with chalky deposits, otherwise same as for *K. ivorensis*.

Minute anatomy:

Same as for *K. ivorensis*.

Material: Syracuse No. 159/3501.

Khaya anthotheca C. DC.

General characteristics of the wood:

Sapwood grayish or light pinkish-red; heartwood rosy-red to very light reddish-brown; lustrous; odorless; medium hard and medium heavy (Sp. Gr. approx. 0.55) ; medium fine textured; straight to interlocked grained. Growth rings indistinct. Terminal parenchyma absent or very sporadic, when present, often with a concentric row of gum canals. Otherwise similar to *K. ivorensis.*

Minute anatomy:

Similar to that of *K. ivorensis,* with the following exception: large *rays* narrower, ranging from 4-6 (mostly 4-5) seriate.

Remarks: This timber is lighter in color than that of *K. ivorensis,* but is denser and heavier, and rarely exhibits any figure on the quarter.

Material: Krukoff's Nos. 153 and 155 from Cameroon, backed by herbarium material.

Khaya euryphylla Harms

General characteristics of the wood:

Sapwood grayish-white to light pink, narrow, grading gradually into the heartwood; heartwood pale rosy-red to light reddish-brown (considerably lighter in color than that of *K. ivorensis*), usually with a silvery or golden lustre depending on the color; odorless; smooth to the feel; moderately hard; light to moderately heavy (Sp. Gr. 0.42-0.72) ; fine textured; straight to somewhat interlocked grained but rarely showing any figure on the quarter. Growth rings fairly distinct, delineated by a darker zone of fibrous tissue, and occasionally in addition by a straightish line of terminal parenchyma. Otherwise similar to *K. ivorensis.*

Minute anatomy:

Similar to that of *K. ivorensis* with the following exception: *vessels* less evenly distributed, more numerous, occasionally crowded in the inner portion of the seasonal increment, seldom over 200 (av. 140-180) microns in diameter.

Remarks: This timber resembles that of *K. euryphylla* in color, density and weight.

Material: Krukoff's Nos. 83 and 102 from Ivory Coast, 154 and 171 from Cameroon, all backed by herbarium material.

Pseudocedrela Harms

Botanical description.

Trees 20–30 ft., occasionally up to 40 ft. in height, and 1.5–3 ft. in diameter, with a straight or somewhat twisted bole 15–20 ft. in length which bears aloft a rather broad, dense crown. Leaves large (about 1 ft. in length), consisting of 5–9 sets of alternate or sub-opposite leaflets typically featured by undulate margins. Bark characteristic, thick, regularly fissured, ranging from light gray to almost silvery white. Flowers 4–5 merous, in wide cymose panicles. Fruit an elongated 5-celled, 5-valved capsule, the nearly flat woody valves becoming reflexed at dehiscence; seeds winged below, the wing membranous, about 6 cm. long.

Size and distribution.

Several species of *Pseudocedrela* have been described but only one, *P. kotschyi* Harms, is recognized by science. This is very widely distributed in tropical Africa, ranging from the French Sudan through the Gold Coast and Nigeria to the Cameroons and extending thence eastward through Ubangi-

Shari Chad to the Anglo-Egyptian Sudan, Abyssinia, and Uganda. The tree is intolerant and is confined to savannah forest where it occurs singly or in groups. It is quite common in certain portions of its range.

General description of the wood.

Since only one species of *Pseudocedrela* is recognized, data pertaining to this wood are inserted under " Species."

Pseudocedrela kotschyi Harms

General characteristics of the wood:

Sapwood grayish-white to grayish-brown; transition from sapwood to heartwood abrupt; heartwood dark reddish-brown, often with irregular dark brown and black spots, or streaked; somewhat dull; with aromatic odor when fresh; rather rough to the feel; hard and heavy (Sp. Gr. 0.80–0.86); fine textured; interlocked grained and often exhibiting a distinct ribbon figure on the quarter; somewhat splintery but the finished surface taking a good polish. Growth rings distinct, delineated by a whitish line of terminal parenchyma. Pores scarce to fairly numerous, evenly distributed, solitary or in short radial groups, small (barely visible with the naked eye), often plugged with chalky white deposits or dark brown infiltration; vessel lines visible with the naked eye. Metatracheal and paratracheal parenchyma indistinct. Rays numerous, evenly spaced, plainly visible with the naked eye; ray fleck low and inconspicuous. Ripple marks present or absent, when present, visible with the naked eye, 35–40 per mm.

Minute anatomy:

Growth rings delineated by a 2–12 seriate band of terminal parenchyma.

Vessels solitary, in short radial groups of 2–7, in tangential groups of 2–3, or in small nests, encircled by a 1–3 seriate sheath of parenchyma which is often interrupted by fibers and rays contiguous to the vessel, 11–21 (av. 16) per mm.^{2}; orifices round or oval, the largest 140–160 (av. 100–130) microns in diameter; segments 300–500 microns long, truncate or rarely tailed; lateral walls 6–10 microns thick; perforations simple, round or oval, horizontal or nearly so; inter-vessel pits numerous, crowded, minute, round or polygonal, 2–3 microns in diameter; pits leading to contiguous rays similar to the inter-vessel pits, numerous to each ray cell, small, round, 2–4 microns in diameter, with distinct border; tyloses absent; chalky white deposits and dark red gummy infiltration sparse to copious.

Fibers very fine to fine, moderately thick to thick-walled, inconspicuously arranged in radial rows, septate, gelatinous or non-gelatinous in alternate bands or strictly non-gelatinous, often occluded with reddish-brown infiltration, rounded or angular in the cross section, 12–20 microns in diameter, 800–1000 microns long; walls of non-gelatinous fibers 3–8 microns thick; gelatinous layer, when present, 4–6 microns thick; inter-fiber pits minute, rounded, simple or nearly so; dark reddish-brown infiltration sparse to abundant.

Parenchyma (*a*) paratracheal, (*b*) metatracheal, and (*c*) terminal, in cambiform rows of 3-many units along the grain which are often further subdivided into loculi, each containing a solitary crystal, or undivided and occluded with dark red gummy infiltration: (*a*) *paratracheal* parenchyma abundant, forming a 1–3 seriate sheath which is often interrupted by fibers and rays contiguous to the vessel; cells thin-walled, very variable in size and shape, usually strongly flattened to conform to the vessel wall, with maximum diameter of 12–50 microns, 50–120 microns long; crystals not observed; gummy infiltration present; starch grains not observed; (*b*) *metatracheal* parenchyma sparse to abundant; cells solitary or in small groups which occasionally coalesce to form short,

1-4 seriate bands of metatracheal-zonate parenchyma; cells thin-walled, the largest about twice as wide as the fibers, angular and then frequently rectangular in cross section, 16-30 microns wide, 40-160 microns long; solitary crystals occasional; infiltration rare; starch grains not observed; (c) *terminal* parenchyma in a 2-12 seriate band which is often interrupted by vessel groups which cross the border of the ring; cells similar to those of the " b " parenchyma.

Rays 5-7 per mm., very variable in width, 1-7 (mostly 3-6) seriate, storied or un-storied, heterogeneous, up to 100 microns wide, 35 plus cells and 900 plus microns (av. 12-25 cells and 300-600 microns) in height; " upright " cells 1-several (mostly 1) at each end of the ray (*t*), 40-80 microns high, 20-30 microns wide, 40-60 microns long; " horizontal " cells rounded or oval (*t*), 20-30 microns high, 12-24 microns wide, 60-140 microns long; crystals occasional in the " upright " cells; reddish-brown infiltration fairly abundant; starch grains not observed.

Ripple marks present, traceable to storied rays.

Material: Vigne's No. 1549 from the Gold Coast, backed by herbarium material; Imp. For. Inst., Oxford No. 1575.

SUMMATION OF THE PHYSICAL AND ANATOMICAL FEATURES OF THE WOODS OF THE SWIETENIOIDEAE

Sapwood varying from white, greyish-white, or yellowish-white to light pinkish-red or greyish-brown. Heartwood ranging through shades of pale rosy-red, dark reddish-brown with or without a purplish cast, wine-red, purple, and chocolate-brown; dull to highly lustrous; odorless or with a faint cedary odor; tasteless; soft to very hard; light to very heavy (Sp. Gr. 0.35-1.2); fine to fairly coarse textured; straight or interlocked grained and in the latter instance showing a wide variety of figures on the quarter. Growth rings distinct or indistinct; when distinct usually delineated by a line of terminal parenchyma. Vessels small to large (up to 300 microns in diameter), solitary and in radial groups of 2-10 or less frequently in nests or in short tangential groups, occasionally open but generally wholly or in part occluded with yellowish-white, reddish-brown or blackish infiltration or with chalky deposits, quite evenly distributed (unevenly distributed in *Soymida*), sparse and distant to fairly numerous (0-40 per mm.^{2}) and close; vessel segments truncate or short-tailed, rather short (150-600 microns long); perforations simple, usually nearly horizontal; inter-vessel pits very numerous, minute (2-4 microns in diameter), frequently with confluent apertures. Fibers thin- to thick-walled, septate or non-septate, often gelatinous, 12-34 microns in diameter, 300-2100 microns long. Terminal parenchyma generally well developed (usually absent in *Lovoa* and sporadic in *Khaya*); metatracheal parenchyma sparse to fairly abundant, the cells solitary or in small groups; paratracheal parenchyma sparse to fairly abundant, forming a 1-4 seriate sheath about the vessel orifice; zonate parenchyma (both metatracheal- and paratracheal-zonate) abundant in *Entandrophragma* (except in *E. angolense* C. DC.), occasional in *Pseudocedrela*, wanting in the other genera. Rays 3-8 per mm., 1-10 seriate, heterogeneous or homogeneous, on the basis of size, obviously of two sorts in *Khaya* and *Soymida*, in the remaining genera more or less intergrading in width and hence interpreted as being of one type; infiltration sparse to co-

pious; crystals present or wanting. Ripple marks present or wanting, when present, traceable to storied rays.

Key to the genera of Swietenioideae on the basis of the gross and minute anatomy of the wood

1. Terminal parenchyma absent 2
1. Terminal parenchyma present 3
    2. Heartwood pinkish to reddish-brown. Rays of two sizes, heterogeneous; large rays 4–10 seriate; small rays 1–3 (mostly 1–2) seriate ....*Khaya*
    2. Heartwood greyish-brown to dark chocolate-brown. Rays uniform in width, 2–5 (mostly 4) seriate, homogeneous for the most part ....*Lovoa*
3. Metatracheal- and paratracheal-zonate parenchyma absent 4
3. Metatracheal- and paratracheal-zonate parenchyma present, arranged in continuous or broken, wavy lines ....*Entandrophragma* (except *E. angolense*)
    4. Rays of two sizes; large rays 4–10 seriate; small rays 1–3 (mostly 1–2) seriate (consisting for the most part of "upright" cells in *Khaya*) 5
    4. Rays intergrading in size, 1–7 (mostly 3–6) seriate 6
5. Heartwood pinkish to reddish-brown; wood soft to medium hard, light to medium heavy (Sp. Gr. 0.46–0.75). Terminal parenchyma sporadic. Rays with 1–8 "upright" cells at each end (t) ....*Khaya*
5. Heartwood dark blood-red to dark purple- or chocolate-brown; wood very hard and heavy (Sp. Gr. 1.0–1.2). Terminal parenchyma abundant. Rays generally with only one "upright" cell at each end (t) ....*Soymida*
    6. Heartwood pink to reddish-brown. Rays heterogeneous for the most part (homogeneous to heterogeneous in *Entandrophragma angolense*) 7
    6. Heartwood yellowish-red to yellowish- or olive-brown. Rays homogeneous for the most part ....*Chukrasia*
7. Vessels solitary or in groups of 2–10. Paratracheal parenchyma mostly in 1–2 seriate sheaths. Rays strictly heterogeneous, frequently storied 8
7. Vessels mostly solitary or in radial groups of 2–3. Paratracheal parenchyma in 1–4 seriate sheaths. Rays homogeneous to heterogeneous, unstoried.
                                            Entandrophragma angolense
    8. Rays 1–7 (mostly 3–6) seriate ....*Pseudocedrela*
    8. Rays 1–6 (mostly 3–4) seriate ....*Swietenia*

SUMMARY

1. The results of the present investigation indicate that the woods of the Meliaceae, sub-family Swietenioideae have the following features in common:

a. Vessels arranged mostly solitary or in short radial groups; inter-vessel pits numerous, minute, 3–4 microns in diameter, the apertures of 2-many frequently confluent.

b. Paratracheal parenchyma well developed, forming a 1–4 seriate sheath about the vessel orifice.

c. Terminal parenchyma well defined in all genera except in *Lovoa*, where it is absent unless associated with gum canals, and in *Khaya*, where it is only sporadic.

d. Parenchyma (all types) frequently crystalliferous, the loculi catenate, each bearing a solitary rhomboidal crystal.

e. Fibers septate (non-septate in *Lovoa* and some species of *Entandrophragma*), frequently gelatinous.

f. Rays mostly heterogeneous, this character reaching its highest development in *Khaya, Soymida, Pseudocedrela,* and *Swietenia* in the order named (heterogeneous or homogeneous in *Entandrophragma* and *Chukrasia,* essentially homogeneous in *Lovoa*).

2. The following gross and minute features of the wood are of diagnostic value in separating the genera of this group:

a. Lovoa is chocolate or grayish-brown in color; it is featured further by the absence of terminal parenchyma and septate fibers, and by essentially homogeneous rays.

b. Soymida is aberrant among Meliaceous woods in color (dark blood-red to purple-brown), in its unusual hardness, and in its greater weight (Sp. Gr. 1.0–1.2); in addition it is characterized by rays of two sizes, (*a*) the larger 5–10 seriate and 40–100 cells high; (*b*) the smaller 1–2 seriate and up to 10 cells high.

c. Chukrasia is usually yellowish-red to yellowish-brown; anatomically it is very close to the *Swietenia* species except that the rays are frequently homogeneous or nearly so.

d. The other four genera, namely *Swietenia, Pseudocedrela, Khaya,* and *Entandrophragma,* all with reddish-brown wood, are more difficult to separate along generic lines.

e. Entandrophragma species, *E. angolense* excepted, can be distinguished from the other Swietenioideae through the presence of well developed metatracheal- and paratracheal-zonate parenchyma. Separation of the varieties of *E. angolense,* devoid of metatracheal- and paratracheal-zonate parenchyma, from the other Swietenioideae with reddish-brown woods, is far more difficult; they can easily be distinguished from the *Khaya* species through the presence of well defined terminal parenchyma (usually wanting in *Khaya*), and by rays intergrading in size, with one or without " upright " cells at each end (*t*) (rays of two sizes in *Khaya,* with 1–8 "upright" cells at each end). The following characters can be used fairly closely with reservation in separating *E. angolense* from *Swietenia* species and from *Pseudocedrela:* vessels mostly solitary or in radial groups of 2–3 (occasionally solitary but frequently in radial groups of 2–10 in *Swietenia* and *Pseudocedrela*); paratracheal parenchyma 1–4 seriate (1–2 seriate in *Swietenia* and *Pseudocedrela*).

f. Careful analyses of the words of *Entandrophragma* species bear out the contention that these fall naturally into four distinct groups, corresponding with the four sub-genera established by Harms on the basis of morphological differences.

g. Khaya and *Swietenia* can be separated without question through the following characters:

Terminal parenchyma absent or sporadically distributed and then wanting from a number of consecutive rings, never conspicuous. Rays of two sizes: (*a*) large rays 4–10 (mostly 4–7) seriate, 40–60 plus cells and up to 1500 plus

microns high, with 1–8 "upright" cells at each end (*t*); (*b*) small rays 1–3 (mostly 1–2) seriate, consisting for the most part entirely of "upright" cells. Ripple marks generally absent or if present, sporadic and irregular ....*Khaya*

Terminal parenchyma abundant, well defined. Rays intergrading in size, 1–6 (mostly 3–4) seriate, occasionally up to 35 cells and 1200 microns high, but mostly 15–25 cells and 300–600 microns high, with 1–3 (usually only 1) "upright" cells at each end (*t*). Ripple marks frequently present .....*Swietenia*

h. Pseudocedrela wood is aromatic when fresh; structurally and otherwise it occupies an intermediate position between *Khaya* and *Swietenia.* It can be distinguished from *Khaya* through the presence of well defined terminal parenchyma and by rays which intergrade in width. Apparently the only character of diagnostic value in separating *Pseudocedrela* from *Swietenia* is found in the rays which are 1–7 (mostly 3–6) seriate in *Pseudocedrela,* and 1–6 (mostly 3–4) seriate in *Swietenia.*

3. *a.* Using the presence or absence of terminal parenchyma as indicative of close affinity between the genera of the sub-family Swietenioideae, three groups can be distinguished—viz.: (1) Terminal parenchyma always present—*Swietenia, Pseudocedrela, Entandrophragma, Chukrasia, Soymida;* (2) terminal parenchyma sporadic—*Khaya;* (3) terminal parenchyma absent, unless associated with gum canals—*Lovoa.*

b. On the basis of heterogeneity of the rays, three groups can be distinguished —viz.: (1) Heterogeneous rays always present—*Khaya, Pseudocedrela, Soymida, Swietenia;* (2) rays homogeneous and heterogeneous—*Entandrophragma, Chukrasia;* (3) rays essentially homogeneous—*Lovoa.*

c. From the data enumerated under "a" and "b," when considered in conjunction with other gross and minute characters of the wood, it is evident that *Lovoa* departs consistently from the other genera of the Swietenioideae on the basis of wood characters.

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DESCRIPTION OF PLATES

Photographs in plates 37–39 inclusive were taken at 15 × with a 48 mm. Bausch & Lomb Micro-Tessar, and reduced 1/3. Photographs in plates 40–48 were taken at 75 × with 32 mm. Bausch & Lomb objective and 10 × Leitz periplane eye-piece, and repro-duced without reduction.

PLATE 37

Fig. 1. *Swietenia mahagoni,* transverse section.
Fig. 2. *S. macrophylla,* transverse section.
Fig. 3. *Soymida febrifuga,* transverse section.
Fig. 4. *Chukrasia tabularis,* transverse section.
Fig. 5. *Lovoa klaineana,* transverse section.
Fig. 6. *Pseudocedrela kotschyi,* transverse section.

PLATE 38

Fig. 7. *Entandrophragma angolense* var. *macrophyllum,* transverse section.
Fig. 8. *E. angolense* var. *dolichocarpum,* transverse section.
Fig. 9. *E. angolense* var. *beninense,* transverse section.
Fig. 10. *E. candollei,* transverse section.
Fig. 11. *E. utile,* transverse section.
Fig. 12. *E. cylindricum,* transverse section.

PLATE 39

Fig. 13 and 14. *Khaya ivorensis,* transverse section.
Fig. 15. *K. klainei,* transverse section. Note an indistinct line of terminal paren-chyma.
Fig. 16. *K. senegalensis,* transverse section. Note an interrupted line of terminal parenchyma.
Fig. 17. *K. euryphylla,* transverse section.
Fig. 18. *K. anthotheca,* transverse section.

PLATE 40

Fig. 19. *Swietenia mahagoni,* transverse section.
Fig. 20. *S. mahagoni,* tangential section. Note uniformity in the size of, and the storied arrangement of rays.
Fig. 21. *S. macrophylla,* transverse section.
Fig. 22. *S. macrophylla,* tangential section.

PLATE 41

Fig. 23. *Swietenia humilis*, transverse section.
Fig. 24. *S. humilis*, tangential section.
Fig. 25. *Chukrasia tabularis* var. *velutina*, transverse section.
Fig. 26. *C. tabularis* var. *velutina*, tangential section.

PLATE 42

Fig. 27. *Soymida febrifuga*, transverse section.
Fig. 28. *S. febrifuga*, tangential section.
Fig. 29. *Lovoa klaineana*, transverse section.   Note rows of gum canals.
Fig. 30. *L. klaineana*, tangential section.

PLATE 43

Fig. 31. *Entandrophragma angolense* var. *macrophyllum*, transverse section.
Fig. 32. *E. angolense* var. *macrophyllum*, tangential section.
Fig. 33. *E. candollei*, transverse section.
Fig. 34. *E. candollei*, tangential section.

PLATE 44

Fig. 35. *Entandrophragma utile*, transverse section.
Fig. 36. *E. utile*, tangential section.
Fig. 37. *E. cylindricum*, transverse section.   Note a row of gum canals.
Fig. 38. *E. cylindricum*, tangential section.

PLATE 45 ˙

Fig. 39. *Khaya ivorensis*, transverse section.
Fig. 40. *K. ivorensis*, tangential section.   Note two types of rays, and long strings
of " upright " cells.   •
Fig. 41. *K. ivorensis*, transverse section.   Note gum canals.
Fig. 42. *K. ivorensis*, tangential section.

PLATE 46

Fig. 43. *Khaya klainei*, transverse section.   Note an interrupted row of terminal
parenchyma.
Fig. 44. *K. klainei*, tangential section.
Fig. 45. *K. senegalensis*, transverse section.   Note well defined growth rings.
Fig. 46. *K. senegalensis*, tangential section.

PLATE 47

Fig. 47. *Khaya euryphylla*, transverse section.
Fig. 48. *K. euryphylla*, tangential section.
Fig. 49. *K. anthotheca*, transverse section.
Fig. 50. *K. anthotheca*, tangential section.

PLATE 48

Fig. 51. *Khaya grandifoliola*, transverse section. ʼ
Fig. 52. *K. grandifoliola*, tangential section.
Fig. 53. *Pseudocedrela kotschyi*, transverse section.
Fig. 54. *P. kotschyi*, tangential section.
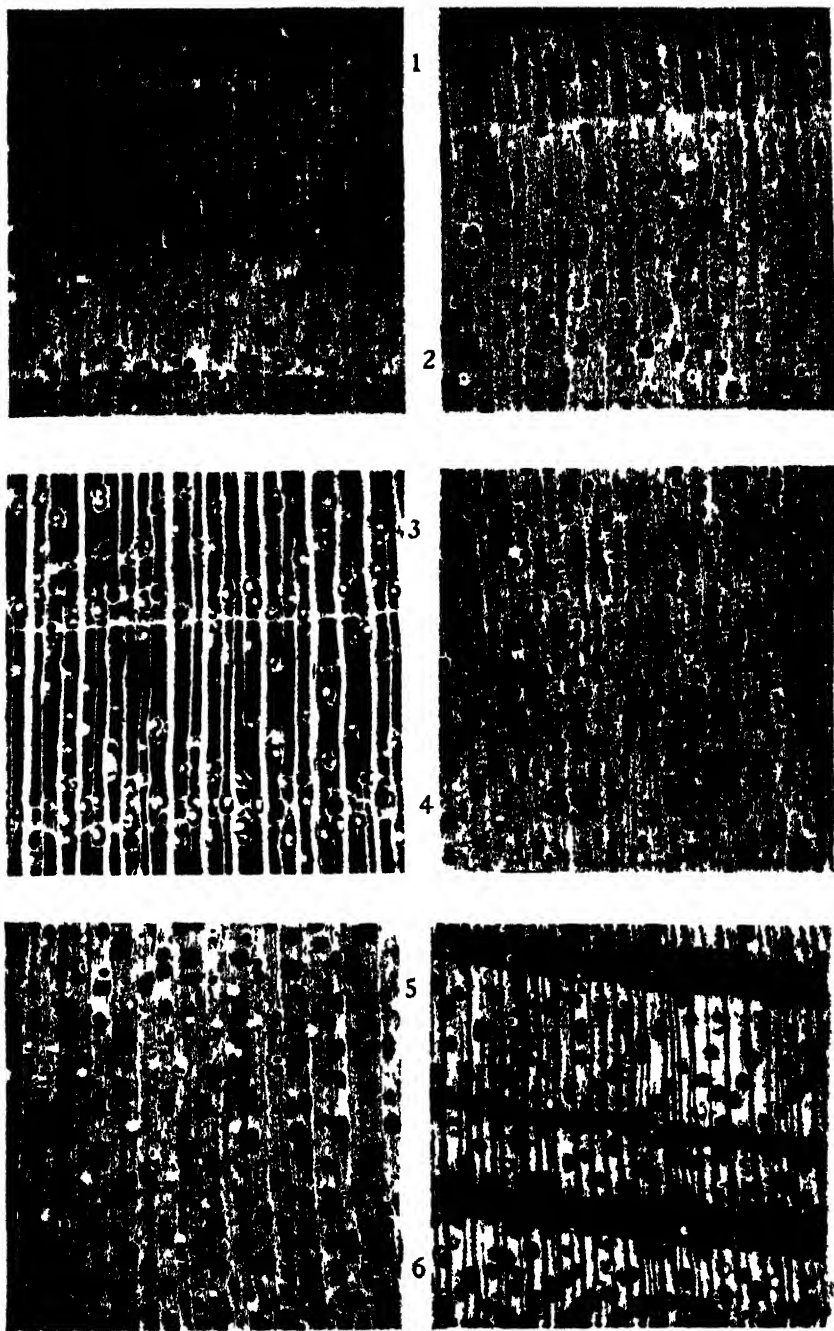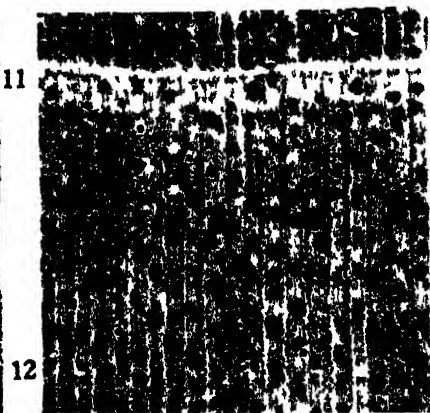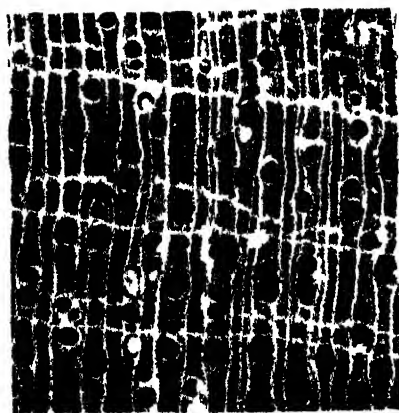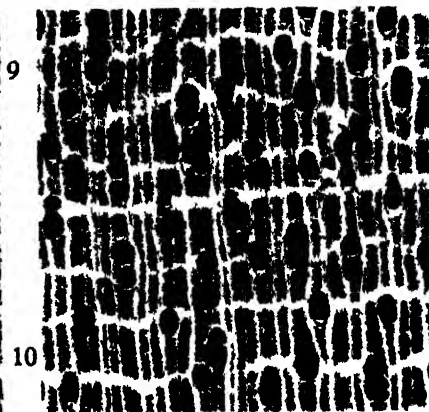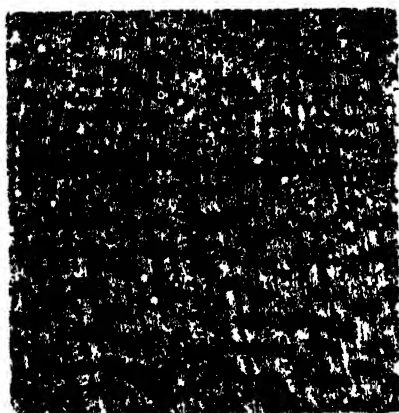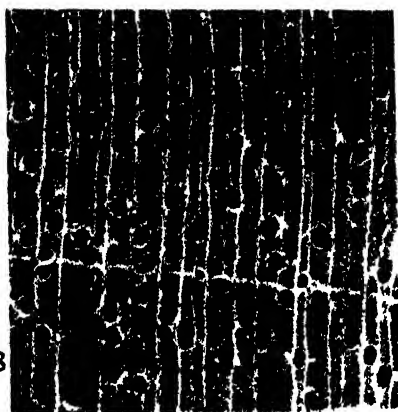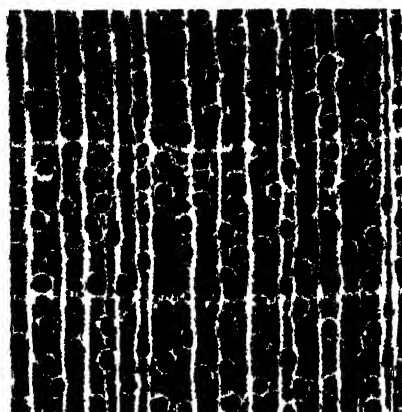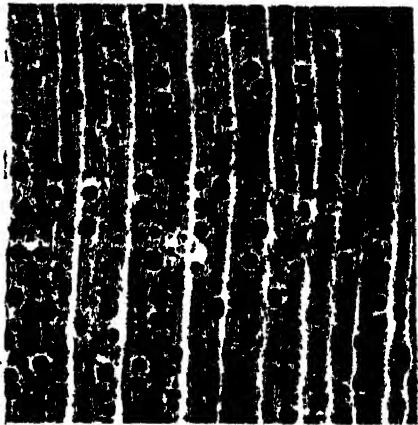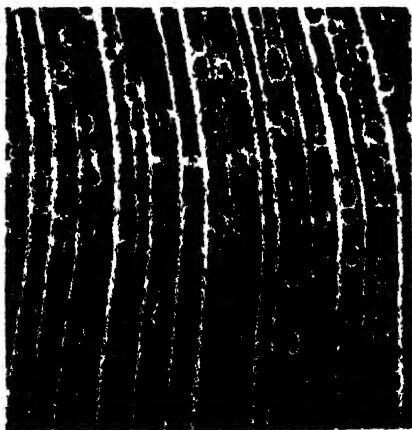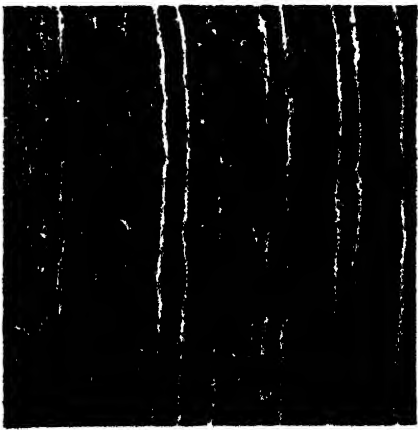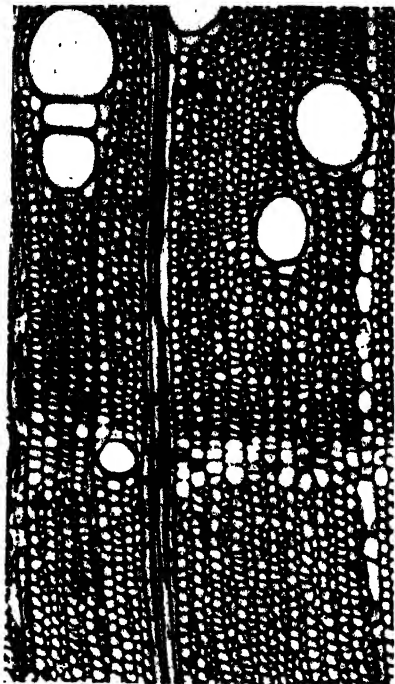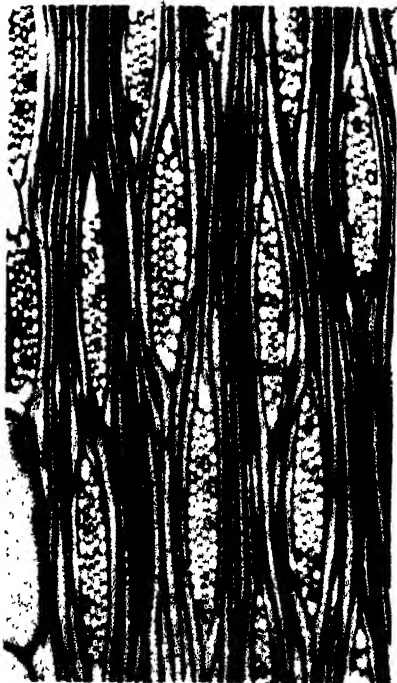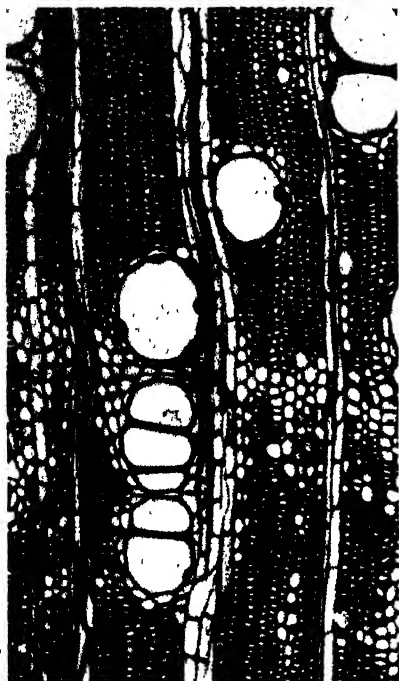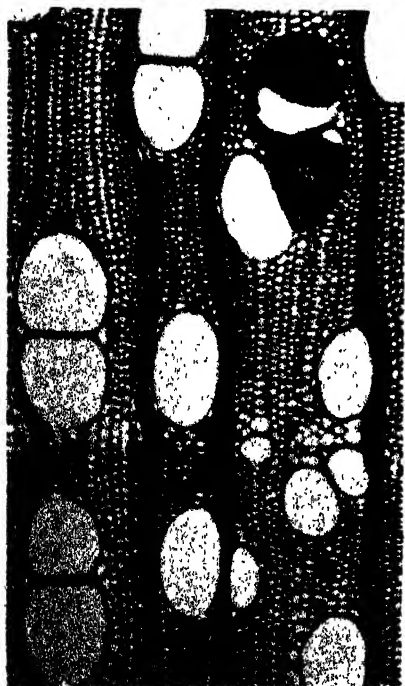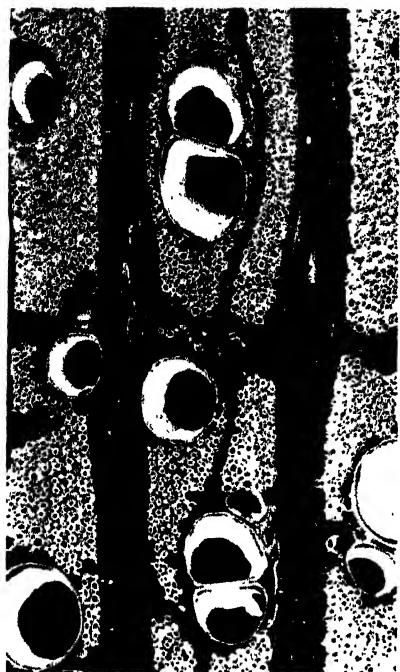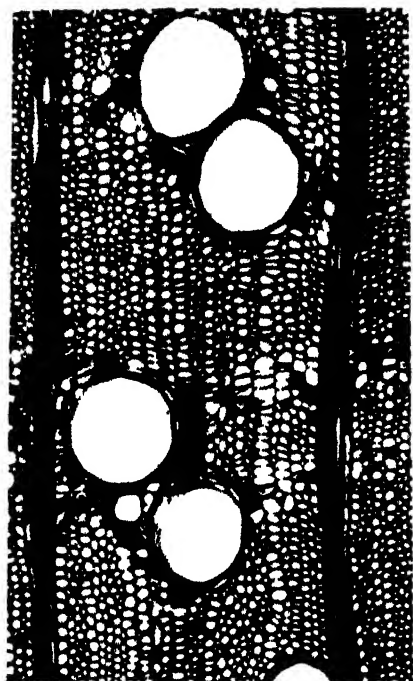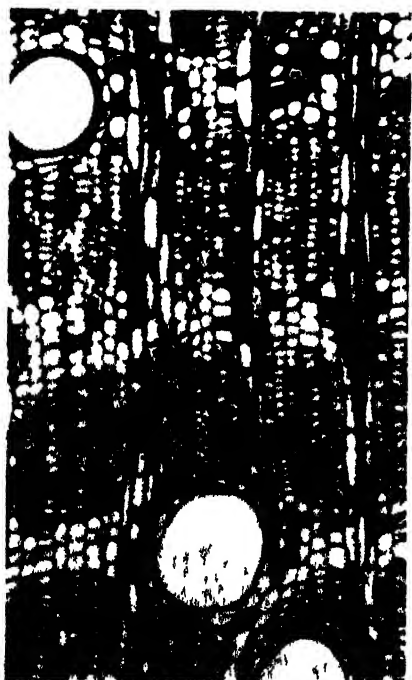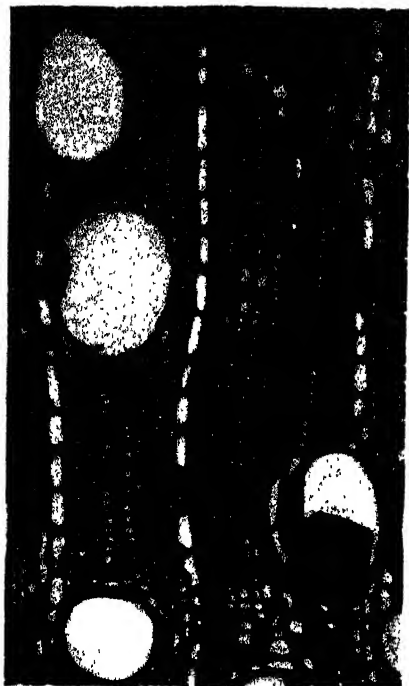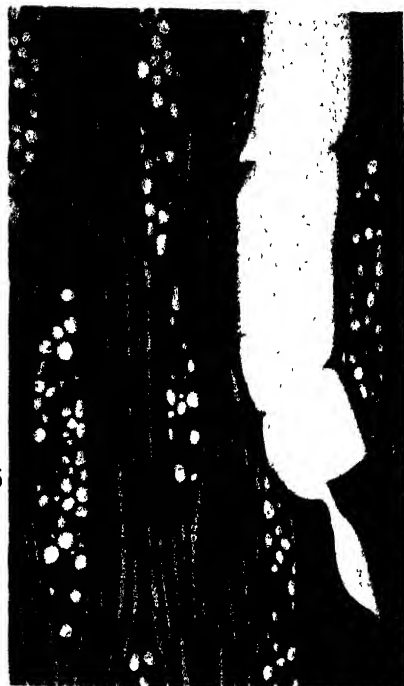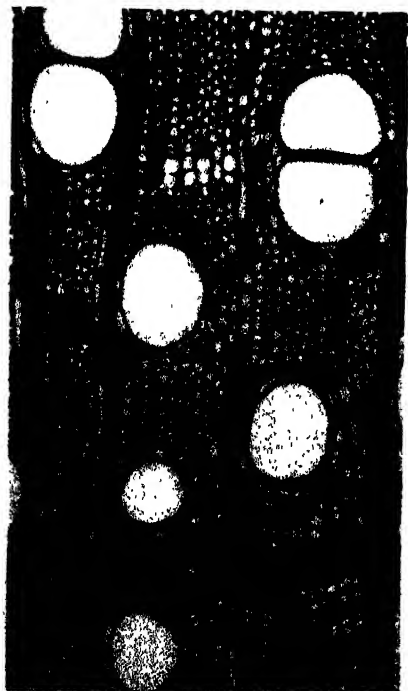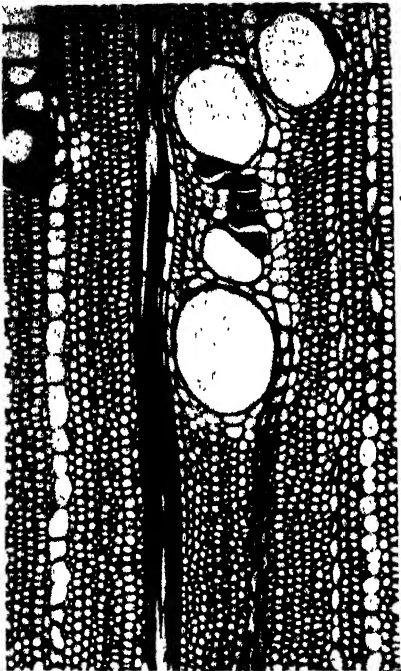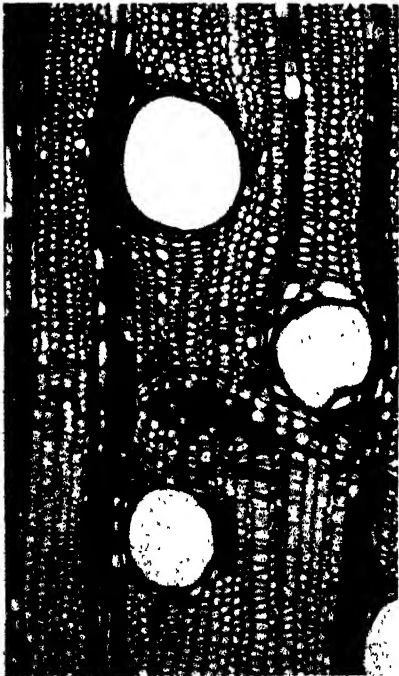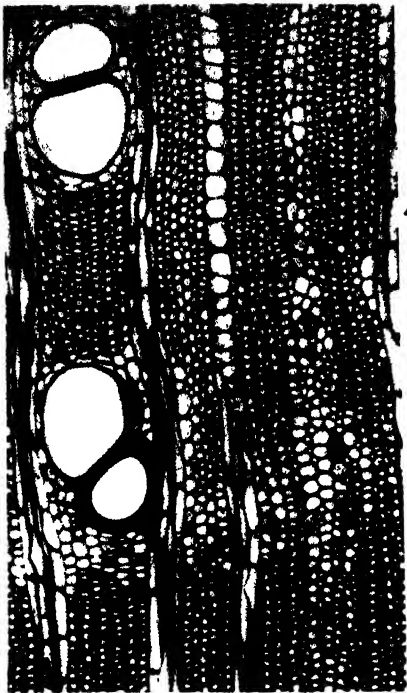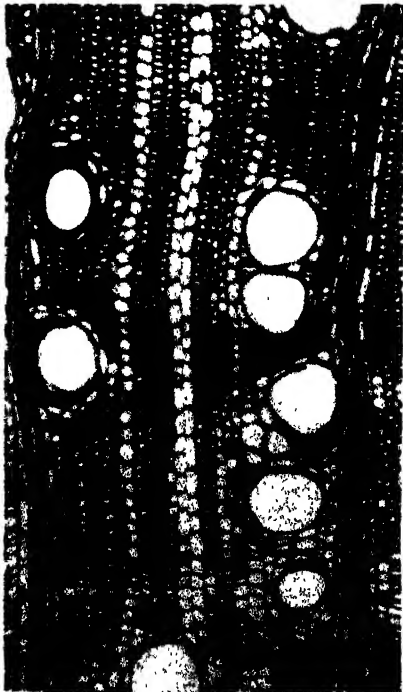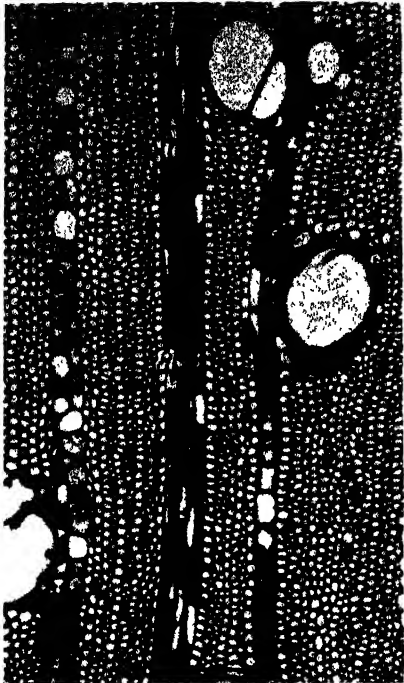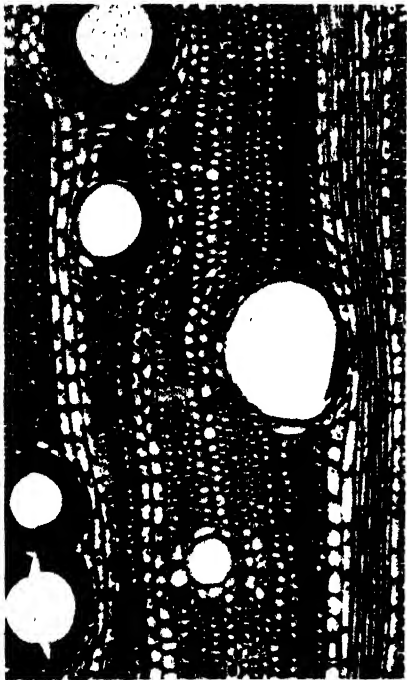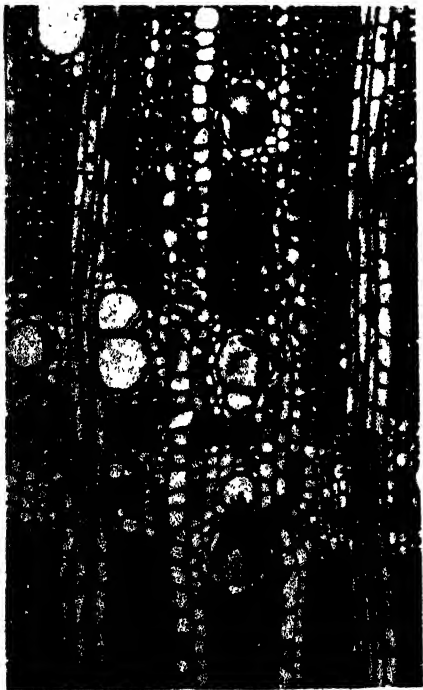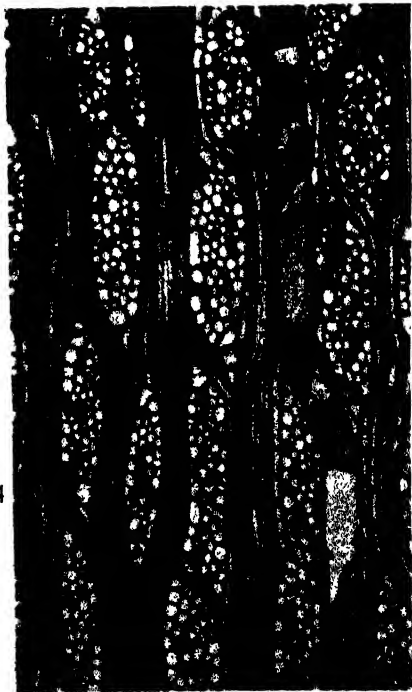
ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, BOSTON, MASS., DECEMBER 28–30, 1933 [1]

The Stimulating Effect of Copper on Chlorophyll Formation. *W. E. Burge, G. C. Wickwire, and O. S. Orth, University of Illinois, Urbana, Ill.*—In a Florida citrus grove, four pounds of copper sulfate were scattered on the ground around each of several three-year-old orange trees whose leaves were spotted yellow or " frenched." In the same part of the grove, several other trees whose leaves were similarly " frenched " were left untreated for controls. Four months later, chlorophyll determinations of the leaves of these two sets of trees were made, using the photo-electric method described by Oltman, and it was found that the leaves of the trees treated with the copper sulfate contained 4.6 times more chlorophyll than did the leaves of the control trees. It should be said in this connection that the copper sulfate greatly improved the condition of the trees. They put on much new growth and the leaves turned green, while the untreated trees put on very little new growth and the leaves remained " frenched." Citrus fruit growers in Florida have always been troubled with the " frenching " of trees set on certain types of soil, and such trees have been successfully treated for the past several years with copper sulfate. Florida cattle, grazing on certain types of pasture land, develop salt sickness, a nutritional anemia, and recently this disease has been cured with copper and iron. It is recognized that copper salts stimulate the production of haemoglobin, the coloring matter of the blood of animals, and it would seem that it also stimulates the formation of chlorophyll, the coloring matter of plants, thus showing a similarity between animals and plants in this respect.

The Wetting Properties of Certain Latex Particles as Shown by the Mudd Interfacial Technique. *Laurence S. Moyer, University of Minnesota, St. Paul, Minn.*—The wetting properties of the latex particles from eighteen species of laticiferous plants (*Asclepias, Euphorbia, Ficus,* and *Musa*) have been investigated in a moving oil-water interface by means of the Mudd interfacial technique. In this technique a droplet of a suitable oil and a droplet of the sol to be investigated are placed together on a microscope slide. A cover-glass is slowly lowered upon them, causing the two droplets

[1] Printed and distributed in advance of the regular issue. Nov. 27, 1933.

to form films which meet in a line across the slide. This interface moves in the direction of the aqueous phase, and particles suspended in the water are thrown into it. Here their behavior can be observed by a high-power, dark-field microscope. The resistance which particles offer to wetting by oil and to deformation by interfacial forces can be noted. From their reactions conclusions concerning the chemical and physical nature of the surface of the latex particles can be drawn. *Asclepias, Ficus,* and *Musa* particles behaved as hydrophilic sols by resisting wetting to a marked degree. *Euphorbia* latex varied greatly from species to species. Species with low latex isoelectric points and non-protein electrophoretic mobility curves showed preferential wetting by the oil. Species with surfaces whose electrokinetic properties corresponded more closely to those of proteins resisted passage into the oil. Evidence has been found for the liquid character of the latex globules of *Ficus elastica* and *Musa ensete.*

Acceleration of Seed Germination with Several Gases. *C. G. Deuber, Yale University, New Haven, Conn.*—The results of germination studies of various seeds when treated with ethylene, vapors of ethylene chlorhydrin, and two illuminating gases will be reported.

Further Experiments on the Effect of Chemicals on the Respiration (CO₂ Output) of Potato Tubers. *Lawrence P. Miller, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Tests with a large number of the mono- and di-substituted halogen derivatives of the lower members of the paraffin series of hydrocarbons showed that many of these compounds increase the CO_2 output of potato tubers several hundred per cent when whole tubers in closed containers are subjected to the vapor of 0.05 cc. of the chemical per liter of air space for 24 hours. *n*-Propyl bromide and *n*-butyl bromide are considerably more effective than the corresponding secondary bromides. The normal butyl and amyl bromides and iodides bring about larger increases than the corresponding chlorides. Methyl, ethyl, and isopropyl alcohols depress the CO_2 output; the four butyl alcohols bring about slight increases. When tubers are treated with ethylene chlorhydrin vapor (which causes very large increases in respiratory activity), an increase in CO_2 output first becomes evident about 12 hours after the beginning of treatment. Shorter periods of treatment also result in eventual increases in respiration. The decrease in CO_2 output resulting from treatments with ethyl alcohol takes place within two hours after the start of treatment.

Factors Affecting the Rest Period of Plants. *W. E. Loomis, Iowa State College, Ames, Iowa.*—Temperature has been found to be the environmental factor having the greatest effect upon the rest period of plants. Depending perhaps upon the conditions under which the species has developed, various resting plant organs have been found to pass through their normal rest

period in minimum time at temperatures either 15°C. above or 15°C. below normal (20°C.). Two varieties of Irish potatoes, six of gladiolus, and one of freesia have been forced by storage or soil temperatures in the neighborhood of 35°C. Under appropriate conditions these same plants may be forced by chemical treatments of the ethylene type. Three varieties of onions, two of tulip, and one of narcissus have been forced by storage or growing temperatures in the neighborhood of 5°C. These plants have not responded to chemical treatment. The chemical responses in the storage tissues have been similar for all successful treatments, whether high temperature, low temperature, or chemical, and have been characterized by accumulations of available carbohydrates, particularly sucrose, in the treated tissues. Nitrogen analyses have shown a tendency toward a reduction of non-colloidal, organic nitrogen with successful treatments.

The Effect of Ethylene Chlorhydrin on the Acid-Base Metabolism of Potato Tubers in Relation to the pH of the Expressed Juice. *John D. Guthrie, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.* — The expressed juice of potato tubers that had been treated with ethylene chlorhydrin and the juice of untreated tubers were analyzed with the object of finding a chemical basis for the higher pH value of the juice of the treated tubers. It was found that the juice of the treated tubers contained less citric, sulphuric, and nitric acid than the juice of the untreated tubers. The amount of $N/10$ acid or base required to produce the pH change was determined from titration curves of the treated and untreated juices. A comparison of this value with the sum of the decreases of citric, sulphuric, and nitric acid expressed in the same terms showed that these changes were adequate to account for the pH change. Expressed in terms of $N/10$ acid, the citric acid decrease is the largest and accounts for the major part of the pH change. The decrease in citric acid is not accompanied by a decrease in the buffer capacity of the juice. This is explained by the development of some substance which compensates for the buffer action of the citric acid. This may be an amino compound, since the treated juice is significantly higher in amino nitrogen. The ash constituents remain practically constant, although the treated juice contains slightly less calcium and magnesium.

A Computation Concerning the Importance of Respiration Water to Young Wheat Seedlings. *Burton E. Livingston, Johns Hopkins University, Baltimore, Md.* — An attempt is made to estimate what portion of the water incorporated by tissue enlargement may possibly be derived from respiration rather than from absorption. Seedlings with coleoptiles just emerged were grown for 46 hours under dilute Shive solution, through which one of a large number of specified gas mixtures continually bubbled. The mixtures contained from 0.6 to 98.3 per cent of O_2, by volume, and each mixture was tested at five temperatures, from 10° to 30°C. It is assumed that the chemical unit $C_6H_{10}O_5$ was ultimately used up and that it was

completely oxidized, which implies that respiration water had about 1/3 the weight of the measured CO_2 produced concomitantly. Estimates of volume increase through enlargement are derived from shoot measurements made at the end of the period and from a subsequent study of the relations of shoot and root volume and shoot length. The relative importance of respiration water is estimated by expressing the computed weight of respiration water, in milligrams, as a percentage of the corresponding computed volume increment, in cubic millimeters, it being assumed that volume increment was due almost wholly to water incorporated into the growing tissues. For some temperature-gas combinations giving relatively slow growth and rapid CO_2 (and H_2O) production, respiration water is estimated as amounting to as much as 8 per cent of the growth water; for other combinations this percentage is as low as 1.0, or even lower. Its magnitude varies inversely (but not proportionately) as does growth efficiency with respect to carbon waste.

The Formation of Cellulose Membranes by Microscopic Particles of Uniform Size in Linear Arrangement. *Wanda K. Farr and Sophia H. Eckerson, U. S. Department of Agriculture and Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—In the cytoplasm of young cotton fibers particles of cellulose are present singly and in bead-like strands. The optical and microchemical properties of the single particle establish its cellulose nature and reveal the presence of a thin layer of pectic substance upon its surface. A single strand of these pectic-coated cellulose particles forms a fibril of the mature fiber wall. In fibers from the unopened boll in which the pectic material is moist, and in dehydrated fibers through the action of certain mild pectic solvents, the fibrils are readily separated with no apparent distortion. These single fibrils, through the additional influence of slight mechanical treatments, separate transversely into microscopic particles of uniform size which are identical with those present in the cytoplasm of the young fiber. The rôle which they play in the building up of the fiber wall presents a new conception of the method of formation of cellulose membranes in plant cells. Comparative studies were made upon certain bacteria, algae, fungi, and mosses as well as upon higher forms closely related to cotton.

Composition of the So-Called Middle Lamella in the Cambium and its Lignified Derivatives. *Thomas Kerr and I. W. Bailey, Bussey Institution, Harvard University, Boston, Mass.*—The so-called middle lamella of lignified tissues is not composed solely of lignin as has been postulated from investigations, based largely upon the action of 72 per cent H_2SO_4. When sections are treated with 72 per cent H_2SO_4, there remains the lignin residue of the intercellular substance, of the original cambial or primary walls, and frequently that of the outermost layer of the secondary walls. The cambial or primary walls, containing cellulose, persist in the adult tissues as a thin, heavily lignified layer, which gives the structural appearance to the acid

residue. In the cambium there exists material within and between the primary walls, which has the properties of pectic compounds. In the mature wood the region originally containing these pectic compounds is the most heavily lignified. Nevertheless, delignification and maceration are not necessarily coincident reactions, and when sections are carefully treated with chlorine water and ammonia, the cells are still bound together, although they may be macerated by pectic solvents.

The Nature and Distribution of Plasmodesma in the Tobacco Plant. *L. G. Livingston, Bussey Institution, Harvard University, Boston, Mass.*—The nature and distribution of plasmodesma in the tobacco plant were investigated preliminary to an attempt to ascertain their possible rôle as a path for the transfer from cell to cell of the virus agency causing the mosaic disease of tobacco. The threads appear to be protoplasmic in nature, and the results of the present researches indicate that they are present throughout all living tissues of the tobacco plant, although certain of the tissues have not as yet been subjected to a critical examination. Determinations of their numerical distribution per unit area of the cell walls of various tissues indicate that there is a wide difference in the number of threads present in the various tissues and between the various tissue systems of the plant, although the numerical distribution in any given tissue is relatively constant. The transfer from cell to cell of the virus principle causing tobacco mosaic via the plasmodesma has not been definitely established, although it seems very probable that transfer by this agency may be a logical deduction. Inoculation experiments indicate that the virus principle, if introduced on the surface of the plant, will not enter uninjured cells; and it is difficult to conceive of the migration of such a substance from cell to cell through homogenous cellulose walls.

Some Effects of Radiation from a Quartz-Mercury Arc upon the Mineral Composition of Plants. *W. D. Stewart and J. M. Arthur, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Several species of plants were grown under shading cloth, under glass, and in the open, and irradiated with a quartz-mercury vapor lamp. Tomato, tobacco, lettuce, and salvia plants in soil exhibited an increase in ash upon irradiation when grown under conditions of low light intensity; tomato plants in soil failed to show such an increase upon irradiation when grown under conditions of high light intensity. Tomato plants grown in sand supplemented with a nutrient solution deficient in calcium displayed an increase in ash after irradiation under conditions of both high and low light intensity. The increase in ash was accompanied by an increase in calcium or phosphorus, or both. Magnesium and manganese contents were unaffected by irradiation. Increase in ash was independent of apparent injury to the plant, and appeared forty-two to seventy-two hours after exposure under the lamp. Use of glass filters showed the wave lengths effective to be within the range 2900 to 3130 Å.

Cabbage, which has been shown to possess no antirachitic value even after exposure to ultra-violet, failed to give an increase in ash upon irradiation. Preliminary experiments with plants treated with irradiated ergosterol indicated the possibility of a connection between response to irradiation and activation of ergosterol present in the plant.

Injury to Plants from Vapors of Mercury and Compounds of Mercury. *P. W. Zimmerman and William Crocker, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Vapors from metallic mercury and compounds of mercury applied to soil are toxic to plants. The dangers from treating greenhouse soil with mercury compounds were shown by the fact that when the soil of one bed of roses was watered with a 0.05 per cent solution of bichloride, the flower buds of Briarcliff roses over the entire range were injured. Peduncles turned brown and half-mature buds opened with faded, brown petals. This type of injury was induced also when potted plants were enclosed in a case with metallic mercury or rich soil moistened with 0.1 per cent solution of bichloride. Leaves also were susceptible to injury but were more resistant than flower buds. Chemical analysis of gardenia leaves from plants enclosed in a case three days with treated soil showed 1310 ppm. of mercury on a dry-weight basis. When air was drawn through treated soil and then over gold leaf, mercury amalgam was formed. The mercury then liberated by heat reacted with iodine, forming red mercuric iodide. When bichloride crystals were substituted for treated soil, no mercury amalgam was formed. Mercurous chloride crystals were ineffective until mixed with moist soil. Seventy plant species were injured by vapors from mercury or compounds of mercury, though variation in susceptibility was evident, five being quite resistant. Six organic and eight inorganic mercury compounds applied to soil containing organic matter were effective. The effectiveness was greatest at 75°F. or above and decreased with the temperature to 40°F., where no injury occurred within seven days.

Toxic Action in Soil of Illuminating Gas Containing Hydrocyanic Acid. *A. E. Hitchcock, William Crocker, and P. W. Zimmerman, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—The toxic action of illuminating gas in soil as compared to its effect when confined only with the aerial parts of plants was distinctly different with respect to the mimimum amount of gas causing injury, the principal toxic constituent, and the type and degree of injury. Under certain conditions the residual products of illuminating gas left in the soil were more toxic than the flowing gas. Storage of gassed soil in the open for 1 to 5 days was more effective in removing the highly toxic substances than leaching the soil with water. Since loss of toxicity from sealed samples of gassed soil was more rapid at room temperature than at 3°C., it would appear that the change in toxicity might be of a biological nature. With short periods of exposure and a rapid flow of gas, roots of potted plants were injured or killed and the aerial parts

eventually exhibited injuries that were indistinguishable from those caused by high temperature or lack of water, or both. Under these conditions hydrocyanic acid was the principal toxic constituent. Illuminating gas was rendered approximately 25 times less toxic when HCN was removed by scrubbing the gas with alkali hydroxide or silver nitrate. Carbon monoxide, ethylene, acetylene, propylene, and butylene were not injurious when used in amounts equivalent to their occurrence in minimum toxic amounts of illuminating gas. Lack of oxygen was not a limiting factor in these experiments.

Starch Determinations with Takadiastase. *F. E. Denny, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Confirmation was obtained of previous claims that potato starch is hydrolyzed completely to glucose provided that enough takadiastase is added. The pH range is not narrow but may vary from 3.5 to 5.0. A long period of boiling of the starch paste previous to adding the takadiastase is unnecessary; however, the temperature of the water must reach at least 70°C. at the time the starch is added. In determining starch in plant tissues, takadiastase gives high values for the blank, but when dialyzed overnight in collodion bags it is effective in producing complete hydrolysis and gives zero readings with Fehling's. Starch determinations on various plant tissues using takadiastase and omitting subsequent acid hydrolysis gave values agreeing with the Walton and Coe Official Method. But evidence was obtained that the values by both methods are too high, that there is a lack of specificity for starch, and that accompanying non-starch substances are included and estimated as starch.

A Method for Determining the Relative Humidity in the Intercellular Spaces of Living Plant Tissues. *Luther Shaw, Cornell University, Ithaca, N. Y.*—Need of a method for determining the relative humidity in the intercellular spaces of living plant tissues arose in studies on the nature of resistance and susceptibility to fire blight in apple and pear. The physical relations existing in open systems between osmotic concentration and vapor pressure and between vapor pressure and relative humidity were suggested by Professor O. F. Curtis as offering a basis for calculating the relative humidity in intercellular spaces from the turgor deficit of cells. For example, if a group of cells at 24°C. had a turgor deficit equivalent to a 0.4 weight molar solution of sucrose, the vapor pressure of the cells should be the same as that of the sucrose solution or 22.21 mm. Hg. The vapor pressure of pure water at 24°C. is 22.38 mm. Hg. Therefore, the relative humidity in the intercellular spaces of the tissues would be 22.21/22.38, or 99.2 per cent of saturation. Change in the curvature of rings of cortical and phloem tissues of apple and pear shoots was taken as a criterion for finding the concentration of sucrose with which the cells were in equilibrium   Numerous tests on shoots under various controlled conditions favoring differences

in turgor indicated that relatively accurate measurements of turgor deficits of the cells were obtained by this method and that the calculated relative humidities for the intercellular spaces were approximately correct. These conclusions gained further support in biological tests.

Prosenchyma an Orientation of Parenchymal Cells of Fundamental Importance. *Frederic T. Lewis, Harvard Medical School, Boston, Mass.*— Link introduced the term prosenchyma for polygonal cells with dovetailed ends, and Hayne (1828) nearly appreciated its real significance. "The arrangement of vesicles," Hayne said in a foot-note, "if we disregard the axis of the plant, is essentially the same in both cases, for no other arrangement that would fill space is conceivable." Both in parenchyma and prosenchyma a section of the tissue is primarily a mosaic of hexagons; but in parenchyma the orientation is with *sides* above and below (in the axis of the stem), whereas in prosenchyma, *angles* are above and below. Prosenchyma is parenchyma turned through an angle of 30° (or of 90°, which yields the same result). Let the tension in the walls be greater in the vertical axis of the stem than in the transverse axes. Parenchyma then becomes the muriform tissue of Bernhardi, with cells which are rectangular in vertical section. The same tension converts prosenchyma into fibers pointed at both ends. Elastic models showing these transformations are easily made, and calculations of the relative tensions are in progress. Considered in three dimensions, parenchyma is the orientation of tetrakaidecahedral bodies with *surfaces* above and below (in the axis of growth); prosenchyma is an arrangement of tetrakaidecahedra with *edges* above and below. The distinction is neither " weak " nor " practically useless."

Dormancy in Tilia Seeds. *Lela V. Barton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Dormancy in seeds of *Tilia americana* L. is due to an impermeable seed coat and a partially dormant embryo. The seed coats were rendered permeable by treatment in moist granulated peat moss at 20°C. for four months. An additional five months at 1° or 5°C. served to after-ripen the embryo. This method was applied practically by planting the fruits in flats in June or July and placing the flats in a mulched cold frame during the winter. Inhibition offered by the seed coat was effectively overcome by treating extracted seeds with concentrated sulphuric acid for twenty minutes. Seeds thus treated gave good seedling production after three or four months at 1° or 5°C. Catalase activity and the rate of growth of excised embryos increased with after-ripening. Oven tests and sample plantings as well as plantings made directly in flats using seeds of *T. tomentosa* Moench., *T. platyphyllos* Scop., and *T. cordata* Mill. indicated the same general trend as *T. americana*. However, *T. tomentosa* germinated very poorly throughout the tests, while *T. platyphyllos* and *T. cordata* germinated more easily and with higher percentages than *T. americana*.

Plastid Structure and Its Possible Effect on Photosynthesis. *T. Elliot Weier, Connecticut College, New London, Conn.*—The non-starch-containing plastid presents a homogeneous appearance. Starch granules appear evenly distributed throughout the plastid cytoplasm. As the granules increase in size, it appears in *Polytrichum* that the mass of plastid cytoplasm decreases. Assuming that the rate of photosynthesis depends upon the amount of plastid substance, we should have in this phenomenon an explanation of a number of facts concerning the rate of photosynthesis as well as of Willstätter's " cytoplasmic factor." Numerous observations point to the fact that chlorophyll must exist in the plastid in solution and yet colloidally dispersed. A theory of plastid structure is adduced to explain this and certain other facts noted in connection with plastid behavior. Attempts are then made to fit this theory with other known plastid phenomena and to one of Briggs' recent schemata of photosynthesis.

A Study to Determine the Range of Wave-Length Most Effective in Stimulating Reproductive Growth in Marchantia. *N. A. Schappelle, Cornell University, Ithaca, N. Y.*—*Marchantia polyrhiza* was grown vegetatively to maturity in natural light during the winter months in a greenhouse. From this culture plants were placed into containers covered by lids into which light filters made by the Corning Glass Works were fastened. These filters transmitted narrow ranges of wave length in the visible spectrum. The per cent of visible light transmitted by a certain filter was always very closely equal to the per cent total of infra-red transmitted. The calculated quantities of energy of the respective wave lengths in the visible spectrum that reached the plants under the filters were about in the same ratio as they occur in the energy distribution of an ordinary electric light bulb. The results showed consistently that there is an optimum range of wave length that favors reproductive growth in *Marchantia*. This range is in the orange-red part of the spectrum, $0.65\ \mu$ to $0.75\ \mu$. Any plants not receiving rays of this wave length did not respond. Green, blue, and violet, even at intensities so high as to cause injury, did not cause any noticeable fruiting. The length of day was not all-important, for occasional fruiting was noted in an 8-hour and 10-hour day if given the proper rays. However, if the red-orange rays could act over longer diurnal periods of time, the fruiting was increased. Increasing intensity of these rays to a certain value also increased the response. Low temperatures, low inorganic salt content (especially nitrates) of substrate, and red-rich radiation all seem to favor the formation of fruiting bodies.

Yarovization Formulas for Winter Oats and Barleys. *Dmitry N. Borodin, U. S. Department of Agriculture, Washington, D. C.*—Thirty-five varieties of oats and fifty varieties of barleys were treated to determine yarovization formulas for production of yarovization effect. Under the yarovization formula is understood the predetermined combination of three

factors: moisture, time, and temperature, indicated concisely in a ratio of "A : B : C," in which "A" is moisture p.c. per dry weight of seeds, "B" time of treatment in days, "C" temperature (centigrade). The plants grown from the yarovized seeds in Arlington Experimental Farm, Rosslyn, Va., and in Aberdeen, Idaho, showed characteristic response or yarovization effect in the form of acceleration of heading or full heading in experiment and grass-cluster stage in the check. The seeds sown at Aberdeen, Idaho, were in the mail seven days and covered the distance of 1,979 miles. Their acquired properties of yarovized seeds remained and yarovization effect was reported. Three varieties of Winter Turf type produced full heads in the experiment, but remained in grass-cluster stage in checks. Similar results were obtained in the case of barleys, but the time and temperature of treatment were different. According to their response, oat and barley varieties may be divided into five types: (1) Spring type—heads in experiment and check; (2) spring-winter type—slight acceleration in experiment; (3) winter-spring type—pronounced acceleration in experiment; (4) winter type—heads in experiment and remains in grass-cluster stage in checks; (5) stubborn winter type—the grass-cluster stage in experiment and check (if no re-yarovization is applied). The pre-sowing treatment or yarovization formula necessary to produce the yarovization effect is quite specific for any one variety. Plants of winter oats grown from yarovized seeds are less subject to infection by crown rust (*Puccinia coronata*).

The Effect of Carbon Dioxide and Some Other Gases on the Germination of Seeds of Poa compressa. *Alice M. Andersen, U. S. Department of Agriculture, Washington, D. C.*—In testing the germination of seeds of Canada bluegrass (*Poa compressa*), they are sown in Petri dishes on filter paper moistened with $N/50$ potassium nitrate solution and placed, daily, in the light at 30°C. for 6 hours and in the dark at 20°C. for 18 hours during a period of 28 days. The writer found in previous experiments that removing the glumes, or the use of dilute nitric acid, could be substituted for potassium nitrate in the germination of these seeds. It seems of interest to determine what effect treating the seeds with various gases might have on their germination, both in the light and in the dark. Seeds subjected to various gases for a week or more previous to the above outlined daily light and temperature alternation gave results which would indicate that carbon dioxide is comparable to the control $N/50$ potassium nitrate (85 to 95 per cent germination). Ozonized air, ozonized oxygen, and nitrogen are also stimulating, while air and medical oxygen gave results (40 to 60 per cent germination) comparable to the control with water. In another series conducted in the same way, but in the absence of light, carbon dioxide gave 70 to 85 per cent germination, which is equal to that obtained with the control $N/50$ potassium nitrate. Ozonized air and ozonized oxygen are also stimulating. Nitrogen is only slightly stimulating in the dark, while air and

medical oxygen are comparable to the control with water (10 to 30 per cent germination).

The Location and Concentration of the Virus of Tobacco Mosaic within the Cells. *B. M. Duggar and L. G. Livingston, University of Wisconsin, Madison, Wis.*—Previous work on plant viruses has apparently furnished little direct evidence bearing upon the problems of the distribution and concentration of virus in relation to the various components of the infected host cell. In the present work, while both macro and micro methods were employed, the more decisive results were obtained by means of the Chambers' micromanipulation apparatus, using special pipettes produced (drawn) mechanically by a device designed by the junior author. It was eventually found possible to extract or withdraw from the large hair cells of the upper part of the stems (1) the vacuolar sap (primarily) and (2) the entire cell contents. The indications are that the main virus concentration is in the protoplasm and not in the cell sap. This too was confirmed by an approximate macro method involving separate vacuolar sap extraction. The most striking results were those in which the entire cell contents of hair cells containing inclusion bodies were contrasted with the contents of hair cells from the same plant containing no inclusions. The virus concentration was greatest where the inclusion bodies were present, and it is suggested that the inclusion bodies at least accompany the development of the virus agency in high concentration. Clear demonstration was obtained that the inclusions are fragile structures, readily breaking into granules when touched with the micropipette.

Effect of Boron on the Availability of Iron. *Antonio G. Rodriguez, Cornell University, Ithaca, N. Y.*—Boron, in concentrations above 1 ppm., appears to be toxic to *Spirodela polyrhiza*. Toxicity is expressed by a general decrease in growth, chlorosis, and loss of roots. Addition of potassium tartrate prevents and corrects this chlorosis, and also increases reproduction and size of plants. With algae, improvement in growth is obtained with the addition of tartrate, even in concentrations which should be highly toxic. It has been found that potassium tartrate increases the concentration of available iron. A marked decrease in iron is obtained when boric acid is present. A flocculent precipitate is formed when boric acid is added. The addition of tartrate decreases the precipitate, and citrate prevents the formation of a precipitate. Apparently boric acid inhibits the solubility of iron. This suggests the possibility that the chlorosis and other toxic symptoms may be due primarily to lack of available iron and not directly to boron itself.

The Growth of Yeast in Water Containing Deuterium. *Oscar W. Richards, Yale University, New Haven, Conn.*—A pure strain of *Saccharomyces cerevisiae* Hansen was grown in William's medium made with

deuterium-containing water [2] having a specific gravity of 1.000061 and with ordinary distilled water, conditions otherwise identical. No difference greater than the normal variation was observed between the resulting cultures in number of cells per unit volume, in the percentage of buds, or in the number of injured or dead cells, permeable to methylene blue, during the 143 hours' duration of the experiment. The total volume of the cells when centrifuged into calibrated tubes was 20 per cent greater from the cultures with a greater concentration of the heavier hydrogen isotope, although the mean cell size was only 3 per cent greater than that from the control culture in distilled water. The dry weight was 26 per cent greater, indicating more solid matter in the cells grown with added deuterium, and this is further demonstrated by a 17 per cent increase in the growth measured with a photoelectric nephelometer.[3] The cells were more uniform in size in the deuterium-containing culture than in the culture in distilled water, as the coefficient of variation of the former was 8 per cent less than that of the latter.

Investigations on the Use of Certain Amino Acids by Green Plants. *Lewis Knudson, Cornell University, Ithaca, N. Y.*—The results of various investigations made by various individuals in the past indicate that some of the simpler amino acids may be absorbed directly by higher plants and utilized as a source of nitrogen. The question has been raised also concerning amino acids as a source of carbon. In my own work consideration has been given to both of these phases of the subject. Orchid embryos were used as the plant material because of the fact that their development requires organic carbon. The results with glycine, leucine, and aspartic acid were negative. The plants showed nitrogen starvation, and as a source of carbon these amino acids were without any value.

Effect of X-Rays on Fern Prothalli. *Lewis Knudson, Cornell University, Ithaca, N. Y.*—Many investigations have been made on the effects of X-rays on green plants, with conflicting evidence respecting the retarding and stimulating effects. Abnormalities of growth have been reported and mutation induced. In my own investigations considerable thought was given to the plant material and cultural methods. It was decided finally to irradiate the spores of the fern *Polypodium aureum*. These are available in great abundance at all seasons of the year, and the germination and subsequent growth may be observed microscopically. Pure culture methods were generally adopted. Some of the outstanding results are as follows: (1) It has not been possible to kill the spores even with high treatments. (2) X-rays have no stimulating effect in so far as an increased growth rate is concerned.

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[2] I am indebted to Dr. T. C. Barnes for the electrolytic water containing the deuterium and to Dr. E. G. Ball for determining its density (cf. Ball, E. G., Biol. Bull., 1933, **65**: 371).

[3] Richards, O. W., and T. L. Jahn, Jour. Bact., 1933, *In Press*. For the other methods cf. Richards, O. W., Arch. Protistenk., 1932, **78**: 263.

(3) Cell division has been completely stopped by high treatments, but a massive single cell has been obtained which lives for more than one year. These single cells develop plastids and are green in color. Fat is stored up in these cells in large amounts. (4) Many remarkable modifications in growth have been noted and photographically recorded.   (5) Striking modifications in chloroplasts occur. In some cases the plastids show vacuoles. In other cases the plastids remain grouped about the nucleus instead of being distributed in the entire peripheral layer of cytoplasm. In other cases giant plastids are produced. These are normal in shape but three to four times the diameter of the normal plastids. These giant plastids appear in part to be formed as the result of fusion of plastids and subsequent breakdown, for in the growing region amoeboid-shaped plastids are formed only one or two to the cell, and such plastids may have areas equal to two sides of the cells. (7) A few cases of apogamy have been noted—that is, production of the sporophyte without the usual sexual processes.   (8) As a by-product a pure culture method for the growth of ferns has been developed.   This should find large use in schools and colleges in the teaching of botany and commercially for the propagation of ferns.

The Effect of Small Amounts of Copper on the Growth of Chlorella and Lemna. *E. F. Hopkins, Cornell University, Ithaca, N. Y.*—Experiments were made in an attempt to demonstrate the necessity of copper for the growth of *Chlorella* sp. and *Lemna minor*. In the first series the copper impurities were removed from the culture solution by treatment with a special sample of bone charcoal which shows marked adsorptive properties toward copper. In the second series the culture solutions were prepared from conductivity water and salts recrystallized three times from conductivity water. These solutions showed no test for copper with sodium diethyl dithiocarbamate (Callan and Henderson, Analyst **54**: 650, 1929). Cultures of *Chlorella* and *Lemna minor* were then set up, using these solutions with and without the addition of copper. No increases in growth were observed in either species due to the addition of copper. In the case of *Chlorella*, concentrations of copper of 1:100 million resulted in growth about equal to the controls, while concentrations of 1:50 million and greater caused marked reduction in growth. With *Lemna minor* copper in a concentration of 1:500 million gave growth about like the controls, and 1:50 million and greater resulted in a depression in growth accompanied by symptoms of copper toxicity.

Further Observations on the Physiological Effect of the Heavy Hydrogen Isotope on Spirogyra. *T. Cunliffe Barnes, Yale University, New Haven, Conn.*—In connection with a series of experiments on the biological effect of melted ice water and recently condensed water (cf. Barnes, T. C., 1932, Proc. Nat. Acad. Sci. **18**: 136; Lloyd, F. E., and T. C. Barnes, 1932, Proc. Nat. Acad. Sci. **18**:422; Barnes, T. C., and T. L. Jahn, 1933, Proc. Nat. Acad. Sci.

19: 638), *Spirogyra* was tested in electrolysis water differing from ordinary distilled water by a slightly higher content of deuterium oxide (term deuterium proposed by H. C. Urey, G. M. Murphy, and F. G. Brickwedde, 1933, Jour. Chem. Phys. 1: 512). A previous report (Barnes, T. C., 1933, Jour. Amer. Chem. Soc. 55: 4332) showed that this water of specific gravity 1.000061 (kindly weighed by Dr. E. G. Ball, 1933, Biol. Bull. 65: 371) maintained the filaments for a longer period than ordinary distilled water and that there was much less abscission in the "heavy" water. In a representative experiment with 90 cc. of distilled water in a 250-cc. Pyrex beaker, the accumulated abscissed fragments occupied a region $4 \times 1.5 \times 1.8$ cm. on the side away from the light, while the filaments in the water containing more deuterium oxide had not abscissed to any extent in the same period (May 11 to June 25, 1933). It is possible that an explanation may involve the bound or more dense water in colloids. The distilled water in the previous controls was redistilled once from Pyrex, but the results have now been duplicated in water distilled as many times (6) as the "heavy" water in the same still. Determinations by Dr. T. L. Jahn with a glass electrode indicated a pH of 6.77 for the "heavier" water, which necessitated buffering. My suggestion that water containing very weak concentrations of the heavy hydrogen will give more significant results than the concentrated H^2H^2O found to be lethal by G. N. Lewis (1933, Jour. Amer. Chem. Soc. 55: 3503), and by H. S. Taylor, W. W. Swingle, H. Eyring, and A. H. Frost (1933, Jour. Chem. Phys. 1: 751) has been recently confirmed by my colleague, Dr. O. W. Richards, who kindly consented to repeat my tests with his yeast cultures (abstract elsewhere in the *Journal*). I am indebted to Prof. H. Castle for advice concerning the plant material. Further experiments with *Oscillatoria* and regenerating planarians are under way.

Physicochemical Reactions in the Tissues of Ripe Pineapple Fruits. *C. P. Sideris and B. H. Krauss, University of Hawaii, Honolulu, Hawaii.—* The tissues of ripe pineapple fruits vary considerably in such physical properties as translucence and specific gravity, and in chemical ones such as acidity and sugar content. On the basis of physical variations, fruits may be classified either as translucent or opaque, or as heavy or light. The development of translucence results from the elimination of gas bubbles in the intercellular spaces of the tissues and their replacement by liquid, which process increases also the specific gravity of the tissues. Unripe fruit tissues are always opaque. From the point of chemical variations, tissues may be either very acid or only slightly acid, the acidity varying usually between 0.2 and 1.2 per cent of citric acid. Opaque tissues are more acid than translucent ones, their higher acidity being supposedly due to improper ripening, as with proper ripening and development of translucence organic acids undergo partial decomposition. The mean of the per cent of citric acid of opaque, semi-opaque, semi-translucent, and translucent basal tissues of summer-

grown clons is 0.998, 0.768, 0.642, and 0.409, respectively, and that of the per cent of sugars 14.82, 15.15, 15.28, and 15.22, respectively.   The percentage distribution of fruits of the different classes according to their specific gravity as determined by their flotation in a 3 per cent sodium chloride solution is as follows: opaque 94.7, semi-opaque 86.5, semi-translucent 12.9, and translucent fruits 5.3.   A similar distribution as determined by the sinking of such fruits in the same solution is as follows: opaque 5.3, semi-opaque 13.5, semi-translucent 87.1, and translucent 94.7.

INDEX TO VOLUME 20

(New names and final members of new combinations are in **heavy-face** type.)